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OBSERVATIONS OF THE NATURE OF THE LIPIDES OF WHEAT FLOUR, GERM AND BRAN

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The damaging effect of unsaturated fatty acids upon gluten quality has been described in a previous communication (Barton-Wright, 1938). The presence of these acids may be correlated with flour grade. In order to enquire into this latter possibility, a patent and two low-grade flours (of 1½% and 12½% length, respectively), bran and germ from the same mill feed, all taken off the mill at the same time, have been examined from the following point of view: if different fatty acids, *or* the same fatty acids in different proportions, occur in bran, germ and flour oils, then it might be possible to determine the degree of milling refinement and the relative amounts of germ and bran contamination present in flours of various grades by examination of the composition of the petrol-ether extracts. For example, how much of the relatively high oil content of low-grade flour is due to flour oil, bran oil and germ oil, respectively?

For the purpose of this investigation the various fractions, *i.e.*, germ, bran, low grades and patent flours, were first of all extracted in 28-pound charges at a time in a large, specially designed Soxhlet extractor with petrol ether for 5 hours. The extracts were evaporated under reduced pressure in an atmosphere of nitrogen to prevent oxidation of the fatty acids. The residue was taken up in more petrol ether and four volumes of acetone were then added to precipitate compound lipides (*e.g.*, phosphatides, glycolipides, etc.). After standing over night at 0° C., the supernatant liquor was filtered off and the "phosphatide" fractions further purified. The amounts of oil and phosphatide in the different fractions are given in Table I.

The percentage of the petrol ether extract in this particular sample of germ proved to be low. It is more usual to find a value of 8 to 10% or more for the petrol ether extract of germ. The values of the petrol ether extracts of the remaining fractions, *i.e.*, bran, bottom

TABLE I

PETROL ETHER EXTRACTS (INCLUDING ACETONE-SOLUBLE AND INSOLUBLE FRACTIONS) OF GERM, BRAN, LOW-GRADE AND PATENT FLOURS

	Total petrol ether extract	Acetone insoluble fraction	Acetone soluble fraction
	%	%	%
Germ	6.716	0.434	6.282
Bran	3.031	0.276	2.765
Bottom 1½% flour	2.840	0.214	2.626
Bottom 12½% flour	1.549	0.187	1.362
Patent flour	0.907	0.183	0.724

1½%, bottom 12½% and patent flours, are normal. It will be observed that the compound lipide fraction thrown down by the addition of acetone decreases in amount from germ to patent flour.

Examination of the Petrol Ether Extract of Germ

Up to the present the petrol ether extract of germ has been most completely examined for the ordinary constants for lipides. The removal of the relatively small amount of compound lipide (= approximately 6½% of the total extract) with acetone did not significantly alter the chemical constants of germ oil, with the possible exception of the iodine value, which was always found to be slightly higher in the acetone-soluble fraction of the oil. The various constants are given in Table II. If the percentage of saturated and unsaturated fatty acids present in a mixture of these substances be known, it is possible

TABLE II

VARIOUS CONSTANTS OF TOTAL PETROL ETHER EXTRACT AND ON ACETONE-SOLUBLE FRACTION OF ETHER EXTRACT OF WHEAT GERM

	Total petrol ether extract	Acetone-soluble fraction
Acid value	24.57	24.40
Saponification value	184.00	186.20
Iodine value	127.40	130.90
Thiocyanogen value	73.48	73.52
Hexabromide value	2.96	2.96
Unsaponifiable residue (%)	4.71	5.21
Saturated acids[1] (Twitchell)	16.00	16.00
Unsaturated acids[1]	84.00	84.00
Iodine value of total fatty acids	134.10	132.90
Thiocyanogen value of total fatty acids	80.87	79.64
Iodine value of unsaturated fatty acids	160.10	158.40
Molecular weight of total fatty acids	278.00	278.00
Molecular weight of saturated fatty acids	266.00	266.00
Molecular weight of unsaturated fatty acids	309.00	307.00

[1] Expressed as a percentage of the total mixed acids.

to calculate from the iodine and thiocyanogen values the percentages of single, double and triple-bonded acids present in such a mixture. The method depends upon the fact discovered by Kaufmann and Keller (1929) that free thiocyanogen $(SCN)_2$ absorbed by a fat or oil does not always coincide with the iodine value, for, unlike iodine, thiocyanogen is not absorbed by all double bonds of unsaturated acids. For example, with oleic acid the single double bond becomes saturated, with linoleic acid and its glyceride the thiocyanogen radicle is absorbed only at one of the two double bonds, while two of the three double bonds in linolenic acid are saturated during treatment with thiocyanogen. It thus becomes possible to formulate equations connecting the percentages of oleic acid, linoleic acid and linolenic acid with total fatty acids in a mixture of these substances. By means of the following equations:

$$O = (100 - G) - 1.104 \text{ (iodine value—thiocyanogen value)},$$
$$L = (100 - G) - 1.104 \text{ (2 thiocyanogen value—iodine value)},$$
$$Ln = (100 - G) + 1.104 \text{ (thiocyanogen value)},$$

where G is the percentage of saturated fatty acids, O = oleic acid, L = linoleic acid, and Ln = linolenic acid percentages, it becomes possible to calculate the percentages of these unsaturated fatty acids in a mixture.

The accuracy of this method of determining quantitatively the amounts of these unsaturated fatty acids in a mixture has been questioned by Smith and Chibnall (1932), who have pointed out that the validity of the method depends, among other factors, upon the correctness of the assumption of Kaufmann and Keller that the thiocyanogen value of linolenic acid is 183. Smith and Chibnall consider that the method does not give accurate values when used for all mixtures of fatty acids in which β-linoleic and β-linolenic acids are present. From their inability to use successfully this method and from certain other reasoning they make the claim that oleic acid is absent in the lipides of the leaves of cocksfoot (*Dactylis glomerata*), which is certainly a curious result.

It must be mentioned here that unless extreme care is exercised the method does give very variable results. The standard solution of thiocyanogen decomposes very readily and is quite useless after seven to ten days. The presence of a trace of moisture in the reaction flasks spoils the estimation. It may have been due to this latter factor that Smith and Chibnall obtained such variable results in their use of this reagent. When the method was first employed in this investigation a good deal of difficulty was encountered owing to the presence of moisture and eventually the thiocyanogen value was determined by

the method laid down by the Committee on Analysis of Commerical Fats and Oils of the American Oil Chemists Society.

The thiocyanogen standard solution was prepared by dissolving 250 g. of neutral lead acetate in 500 c.c. of water. The lead acetate solution was slowly added to the potassium thiocyanate solution with constant stirring. The lead thiocyanate was filtered off on a Buchner filter and well washed with water, alcohol and ether and the lead thiocyanate was then dried as much as possible by drawing air through it. It was then dried in a vacuum desiccator over phosphorus pentoxide for 10 days before using. Next, glacial acetic acid was dehydrated by refluxing with acetic anhydride. For the actual preparation of the thiocyanogen solution, 50 g. of the lead thiocyanate were suspended in 500 c.c. of anhydrous acetic acid, and 5.1 c.c. of bromine (previously dehydrated over sulphuric acid) were dissolved in another 500 c.c. of anhydrous acid. The acetic acid solution of bromine was then slowly added to the lead thiocyanate suspension with vigorous shaking between additions until the solution was completely decolorised. After all the bromine had been added, the precipitated lead bromide and excess of lead thiocyanate were allowed to settle and the whole was then filtered off as rapidly as possible by suction into a filter flask that had been previously dried for an hour at 105° C. For the actual determination of the thiocyanogen values, 0.1 to 0.2 g. of material was accurately weighed into a 200-c.c. glass-stoppered iodine flask and 25 c.c. of thiocyanogen solution added. The flask was then gently shaken until the oil or fat had dissolved and the mixture was allowed to stand in the dark for 24 hours. It should be emphasized that the presence of moisture is fatal to a determination, and in place of adding 10 c.c. of a 10% solution of potassium iodide prior to titration with thiosulphate solution, 1 g. of solid potassium iodide was added to each flask at the end of the experimental period, and the whole shaken for 2 minutes (3 minutes in the case of the blank); this was followed by the addition of 30 c.c. of boiled distilled water and the mixture was titrated against N/20 thiosulphate in the ordinary way. The use of an N/20 thiosulphate solution in place of the usual N/10 solution was found to give more concordant results. The excess of thiocyanogen should be at least 100% and preferably not less than 150% of the amount absorbed. By the adoption of these various precautions the method gave very satisfactory results.

Using the equations given above, and the figures obtained for germ oil, *i.e.*, iodine value = 134.1, thiocyanogen value = 80.27, and 16.0% of saturated fatty acids in the total fatty acids present, the percentages of unsaturated fatty acids in the total mixture of acids obtained from the wheat embryo are:

Oleic acid = 25.2%,
Linoleic acid = 53.5%,
Linolenic acid = 5.13%.

It was mentioned in a previous communication (Barton-Wright, 1938) that there is another unsaturated fatty acid present in germ oil, which is probably palmitoleic acid, with a single double bond in the molecule, but which has not been sufficiently well characterised at present for complete identification. The amount of this acid will be included in the oleic acid figure given above.

Examination of the Petrol Ether Extracts of Bran, Low-grade and Patent Flours

A preliminary examination only was made of the oils (petrol ether extract) obtained from the bran, bottom 1½%, bottom 12½% and patent flours. The values of the chief constants of the three flour oils are given in Table III.

TABLE III

VARIOUS CONSTANTS OF THE PETROL ETHER EXTRACTS OF BRAN, LOW-GRADE AND PATENT FLOURS

	Oil from				
	Bran		Bottom 1½% flour	Bottom 12½% flour	Patent flour
	A[2]	*B*[2]			
Acid value	87.47	83.16	26.81	26.22	19.25
Saponification value	182.10	181.60	175.60	172.6	164.1
Iodine value	127.10	129.90	116.90	114.9	106.9
Saturated acids[1]	16.0	16.0	16.50	16.1	16.6
Unsaturated acids[1]	84.0	84.0	83.50	83.9	83.4
Iodine value of mixed acids	134.50	134.70	132.7	131.6	133.1
Iodine value of unsaturated acids	152.4	152.7	153.3	153.1	157.8
Molecular weight of mixed acids	315.0	306.0	292.0	306.0	299.0
Molecular weight of saturated acids	279.0	279.0	267.0	266.0	267.0
Molecular weight of unsaturated acids	358.0	346.0	303.0	346.0	334.0
Unsaponifiable residue %	12.10	7.35	4.10	4.10	5.60

[1] Expressed as percentage of the total mixed acids.
[2] *A* = Total petrol ether extract.
[3] *B* = Acetone-soluble fraction.

It should be observed that the acid values recorded here for these different oils, as well as for the germ oil, and in particular bran oil, are higher than those obtained by previous investigators, with the exception of Ball (see Jamieson, 1932), who found the figure to be 21.48 for germ oil extracted with ether. Sullivan and Bailey (1936) obtained the figure 6.95 for germ oil extracted with a mixture of alcohol

and ether, while Jamieson and Baughman (see Jamieson, 1932) using ether alone found the figure to be 7.6. The variability in these figures is in all probability due to the varying length of time the germ happened to be stored before extraction. In our case the extraction was not made until 4 weeks after milling, and in the case of bran oil not until three months later. From the results obtained for the acid values of oils in flours stored for long periods, it was found that there was a progressive increase in acid value with time of storage (Barton-Wright, 1938) due, of course, to progressive hydrolysis of fat into glycerol and free fatty acid. It is probable that still higher values would have been recorded for the acid values of these various oils if the flour fractions had been kept for still longer periods before extraction.

With regard to the other figures, the molecular weight of the saturated acids is the same in all three cases and also the same as in germ oil (266). Presumably, therefore, the same saturated acids are present in these flour oils and in germ oil. The molecular weight of the total fatty acids is the same in the three flour oils within experimental error (292–306), but higher than in germ oil (278). The molecular weights of the unsaturated fatty acids show some variation, 303 for bottom 1½% flour, 346 for bottom 12½%, and 334 for the patent flour, while in germ oil the value was found to be 309. It is doubtful, however, if much significance can be attached to the variations in these figures, more especially as the iodine values are the same in all four cases (153–160) within experimental error. The main difference between these oils and bran and germ oil lies in the iodine value of the oils themselves: bran oil = 127.1, germ oil = 127.4, oil from bottom 1½% flour = 116.9, oil from bottom 12½% flour = 114.9, patent flour oil = 106.9. It is probable that these figures are significant, because in earlier investigations it was always found that the higher the grade of flour the lower was the iodine value of the oil.

On the other hand, the iodine value of the mixed acids isolated from either bran, germ, bottom 1½%, bottom 12½% or patent flour oil is the same (132–136). Presumably the iodine values recorded for the oils themselves must be mainly due to the presence of unsaturated fatty acids and the proportion of these bodies is again the same for each of these various oils (approximately 84%). The question therefore arises as to why there should be this progressive fall in the iodine values of the crude oils from bran and germ oils to patent flour oil.

It was thought that perhaps an examination of the unsaponifiable residue might supply an answer. With the exception of that from

bran oil, the unsaponifiable residues of the remaining oils show no significant differences in general character. The unsaponifiable residue isolated from germ, bottom 1½%, bottom 12½% and patent flour oil was found to consist of an oily yellow fraction and very characteristic feathery crystals. This oily fraction was absent in the unsaponifiable residue isolated from bran oil. In this case the mixture was found to be composed of the feathery crystals described above and a rather waxy, dark-coloured solid.

It was found to be impossible to obtain consistent results for the iodine value of the unsaponifiable residue of any of these oils. The values varied from 100 to 160 in duplicate samples. The same difficulty was found by Sullivan and Bailey (1936a) for the unsaponifiable residue of germ oil, and is due presumably to the fact that since unsaturated hydroaromatic bodies are present in the residue, substitution as well as addition of iodine will take place. Until more data are available concerning the reliability and significance of determinations of iodine values of petrol ether extracts it is not possible to express an opinion as to whether the differences found in iodine values of low-grade flour oils, on the one hand, and patent flour oils on the other are in fact due to the presence of germ or bran oil in low-grade flours, or to other factors. In other words it has not so far been possible, by this method, to determine the germ and/or bran content of low grade flours.

Examination of the Fractions Obtained from Germ, Bran, Low-grade and Patent Flours by Extraction with Benzene-Alcohol after Previous Extraction with Petrol Ether

After extraction with petrol ether, the various residues from the germ, bran, low-grade and patent flours were again extracted with a mixture of 80 parts of benzene and 20 parts alcohol for periods of 36 to 48 hours. It has been shown by Rewald (1936) that a further lipide fraction can be extracted from germ by employing this mixed solvent. He found that the extract obtained in this way is composed of a mixture of phosphatides and true fats. It is necessary to continue the extraction with this benzene-alcohol mixture until the solvent remains colourless. The total benzene-alcohol extracts obtained from germ, bran, low-grade and patent flours in this investigation were evaporated under reduced pressure and the phosphatides were precipitated by the addition of warm acetone to the warm extract and the whole was then allowed to stand over night. This method of extraction with the mixed solvent does not appear to be quantitative as far as flour is concerned, and the duplicate samples were never found to agree very exactly. On the other hand, with germ and bran

the duplicates agreed very well. The values obtained for the different milling fractions used in this investigation are given in Table IV, and

TABLE IV

BENZENE-ALCOHOL EXTRACTS (INCLUDING ACETONE-SOLUBLE AND INSOLUBLE FRACTIONS) OF GERM, BRAN, LOW-GRADE AND PATENT FLOURS

	Total benzene-alcohol extract	Acetone-insoluble fraction	Acetone-soluble fraction
	%	%	%
Germ	2.310	—	—
	2.210	0.990	1.220
Bran	3.020	0.470	2.550
	3.190	0.350	2.640
Bottom 1½% flour	0.772	0.265	0.507
	1.052	0.500	0.552
Bottom 12½% flour	0.815	0.420	0.395
	0.696	—	—
Patent flour	0.637	0.305	0.332
	0.810	0.475	0.335

although a general trend may be observed it will be noticed that the duplicates for the two low-grade flours and patent flour do not agree at all closely. With the exception of the bran, the total benzene-alcohol extract from these various fractions shows, on the whole, a decrease from germ to patent flour. It was thought at first that perhaps the values obtained with this particular sample of bran might have been exceptional, since the petrol ether extract and benzene-alcohol extract were almost identical in amount (3.03% and 3.10%, respectively). A number of brans from different sources were therefore extracted with petrol ether and then with the benzene-alcohol mixture. A few of the values obtained are given in Table V. It will

TABLE V

PETROL ETHER AND BENZENE-ALCOHOL EXTRACTS (INCLUDING ACETONE-INSOLUBLE FRACTIONS) OF VARIOUS SAMPLES OF BRAN

Sample	Total petrol ether extract	Acetone-insoluble fraction	Total benzene alcohol extract	Acetone-insoluble fraction
	%	%	%	%
1	2.65	0.16	3.23	0.45
	2.57	0.14	3.16	0.47
2	3.20	0.15	3.26	0.47
	3.22	0.18	3.30	0.55
3	2.75	0.20	2.75	0.48
	2.70	0.11	2.53	0.48

be seen from these results that the amount of lipide material removed by the benzene-alcohol mixture approximates in amount that extracted by petrol ether as far as the bran fraction is concerned.

Examination of these benzene-alcohol extracts is reserved for a separate paper.

Examination of the Acetone-insoluble Fractions of the Petrol Ether Extracts of Bran, Germ, Low-grade and Patent Flours

The acetone-insoluble fraction obtained by the addition of acetone to the petrol ether extracts of these various milling stocks was further purified by dissolving in anhydrous ether and reprecipitating with acetone (4 volumes). With the exception of the fraction obtained from bran, which will be discussed later, these precipitation products from germ oil, bottom 1½%, bottom 12½% and patent flours were obtained in the crude form as dark solids which oxidised in air to still darker products. The product isolated from bran was almost black in colour.

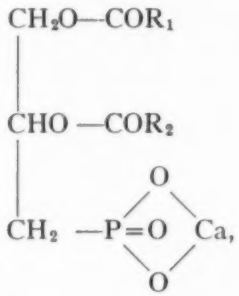
The crude products from germ, bottom 1½%, bottom 12½% and patent flours were tested for lecithin and kephalin after hydrolysing with acid. Three grams of the crude substance were hydrolysed with 5% sulphuric acid for 8 hours, cooled and then extracted with ether. The aqueous solution was reduced *in vacuo* to 100 c.c. and sulphuric acid removed by the addition of baryta. The barium sulphate was removed by filtration, well washed with hot water and lead acetate added to the solutions and washings. A small precipitate formed and this was removed by centrifuging. Excess of lead was removed by means of hydrogen sulphide. The filtrate was again concentrated to 100 c.c. *in vacuo* and then treated repeatedly and alternately with small amounts of a 20% solution of sodium carbonate and a 20% solution of mercuric acetate until a permanent orange precipitate was obtained by the addition of either solution. An equal volume of alcohol was then added and the mixture allowed to stand over night at 0° C. The precipitate was removed by centrifuging, washed with 50% alcohol, suspended in water and decomposed with hydrogen sulphide. The mercuric sulphide was then well washed with hot water, and the washings after acidification with hydrochloric acid were evaporated to dryness *in vacuo*. The dried residue was extracted with alcohol and the alcohol evaporated to dryness on a waterbath. A small residue was obtained. This was tested for β-amino-ethyl alcohol by taking it up in a small amount of dilute hydrochloric acid and adding gold chloride. The mixture was then left in a vacuum desiccator. Brown cubic shaped crystals were deposited after some days. These were recrystallised from water and obtained as long

golden needles, M.P. 189° C. Smith and Chibnall (1932a) give the value for β-amino-ethyl alcohol chloroaurate as 189 to 191° C. Thus there is a small amount of kephalin in the compound lipide fraction isolated from these various oils.

The aqueous-alcoholic filtrate from the mercuric acetate precipitate described above was concentrated *in vacuo* to about 20 c.c. A portion of this was treated with a hot concentrated solution of mercuric chloride. An immediate slight white recipitate appeared and increased in amount on cooling. The melting point of this was 254° C. Smith and Chibnall give the melting point of choline mercuric chloride as 252° C. The presence of choline was further confirmed by adding an alcoholic solution of cadmium chloride to another portion of the solution described above. A small white precipitate formed at once, confirming the presence of choline. Thus both lecithin and kephalin are present in the compound lipide fraction of these oils. The amounts, however, appeared to be small and it was clear that the main bulk of the compound lipide fraction was composed of some other substance or substances than lecithin and kephalin. Incineration gave a large ash which proved to be almost entirely composed of magnesium salts. It was therefore thought possible that the compound lipide fraction might be composed mainly of the magnesium salt of the nitrogen-free lipide, phosphatidic acid, first isolated as the calcium salt by Chibnall and Channon (1927) from cabbage leaves and as the magnesium salt by Jordon and Chibnall (1933) from the seeds of the runner bean, *Phaseolus multiflorus*. This was found to be the case for the products precipitated from the oils of germ, the two low-grade and the patent flours, but not for bran oil. This conclusion was arrived at as follows:

Another portion of the precipitate obtained from the second precipitation with acetone (see above) was first submitted to exhaustive extraction with boiling alcohol. The product was then dissolved in ether and washed with dilute hydrochloric acid. The ethereal layer was reduced by evaporation *in vacuo* to 25% of its original volume and 2 volumes of acetone added. The precipitate that formed was removed and the ethereal solution shaken several times with a saturated aqueous solution of lead acetate. The ethereal solution was well washed with water, concentrated to small bulk and 3 volumes of alcohol added. This resulted in the formation of a precipitate. The precipitate of the lead salt was centrifuged, redissolved in ether and then reprecipitated with alcohol. The lead salt was decomposed with dilute hydrochloric acid, washed with water, and the etheral solution concentrated *in vacuo*. On analysis it gave $P = 4.3\%$ and $N = 0.31\%$. Phosphatidic acid requires $P = 4.52$ and $N =$ nil.

It was found by Chibnall and Channon that the nitrogen content of the product isolated by them from cabbage leaves was only 0.2% and the product could not be lecithin, whereas the atomic ratio of calcium to phosphorus was in all cases very close to unity. On this and other grounds they suggested that the constitution of this substance was that of the calcium salt of a diglyceride-phosphoric acid of the type:



while Jordon and Chibnall (1933) in an investigation of the phosphatides of the seed of the runner bean which has been briefly mentioned above showed that in that seed there is present a mixture of lecithin, kephalin and phosphatidic acid, and that the latter occurs as the magnesium salt. It is only later in the life of the plant that the magnesium salt is converted into the calcium one. It is evident that in the wheat plant a similar state of affairs is in existence. On the other hand, the presence of phosphatidic acid as the main component of the phosphatide-content of plant cells is by no means general. For instance, Smith and Chibnall (1932a) found in the leaves of cocksfoot (*Dactylis glomerata*) that considerable amounts of lecithin and kephalin are present as well as phosphatidic acid, whereas in cabbage leaves the only phosphatide present is calcium phosphatidate.

The acetone-insoluble fraction from the petrol ether extract of bran differed markedly in its properties from those described above for the other milling fractions. It was a dark brown in colour and oxidised in air to an almost black substance. The percentage of nitrogen in the crude product was 2.2% and phosphorus 2.75%. The phosphorus content is low for any of the three phosphatides, lecithin, kephalin or phosphatidic acid. On ashing, a small amount of magnesium was found and considerable amounts (about 1.6%) of iron, but no calcium.

The crude product was purified by the procedure described above and was found to contain considerable amounts of lecithin and kephalin with a small amount of phosphatidic acid as the magnesium salt, as well as an iron compound of unknown constitution.

It would appear from these results that the lecithin-kephalin fraction of the compound lipide content of the wheat grain extractable by petrol ether is mainly confined to the envelope of the grain, whereas in the rest of the grain the lipide is mainly composed of the magnesium salt of phosphatidic acid. A chemical examination has not as yet been made of the products obtained by extracting these various bran, germ and flour fractions with a mixture of benzene and alcohol. This study will form the subject of a separate paper.

Distribution of Fats Throughout the Wheat Grain

It was thought to be of interest to discover the way in which fats are distributed in bran as well as wheat germ itself. For this purpose, portions of unextracted bran, bran extracted with petrol ether and bran extracted with benzene-alcohol were frozen in gum and sectioned on a microtome. The sections were subsequently stained with Sudan IV, which is much to be preferred in this connection to Sudan III, which was found to be very erratic in its action as a stain for fats. For the purpose of staining the bran sections, the technique used by Kay and Whitehead (1935), which was devised for animal tissues, was found to be very satisfactory for plant material if certain modifications were introduced.

A stock solution of Sudan IV (toluene-azo-toluene-azo-β-naphthol) was prepared by adding 2 g. of the dye to one litre of absolute alcohol at room temperature, and then gently boiling until all the powder had dissolved. The solution was allowed to cool and settle. For the actual staining of the sections, 7 parts of the stock alcoholic solution of Sudan IV were mixed with 9 parts of 45% alcohol. The microtomed sections were washed in water for 12 hours to rid them of gum and then transferred to 50% alcohol for 1½ hours. From the 50% alcohol they were transferred to the diluted Sudan IV solution and allowed to remain in it for at least 3 hours at ordinary room temperature; a longer time of immersion does no harm. After the sections had been stained in Sudan IV, they were placed in 50% alcohol for 3 minutes, washed in distilled water for 30 minutes, counter-stained in Mayer's haemalum for at least 3 hours, washed in alkaline tap water and mounted in glycerine.

It will be seen from Figure 1 that the fat in unextracted bran shows as globules in the aleurone layer, and it will also be noticed that the protoplasmic contents of the aleurone cells are stained with Sudan IV, showing that fat is present in the protoplasm as well as separate globules. The testa, *i.e.*, the layer of cells above the hyaline layer, also stains very deeply with Sudan IV, but the pericarp tissues remain clear. Bran which has been extracted with petrol ether shows a

complete absence of fat globules, a result to be expected, but the protoplasm of the aleurone cells still shows fat to be present (Figure 2). As in the unextracted bran, the testa still stains deeply with Sudan IV. After extraction with a benzene-alcohol mixture, it will be seen that the aleurone layer appears to be completely free of fat (Figure 3), but the testa appears to be unaffected and still stains deeply.

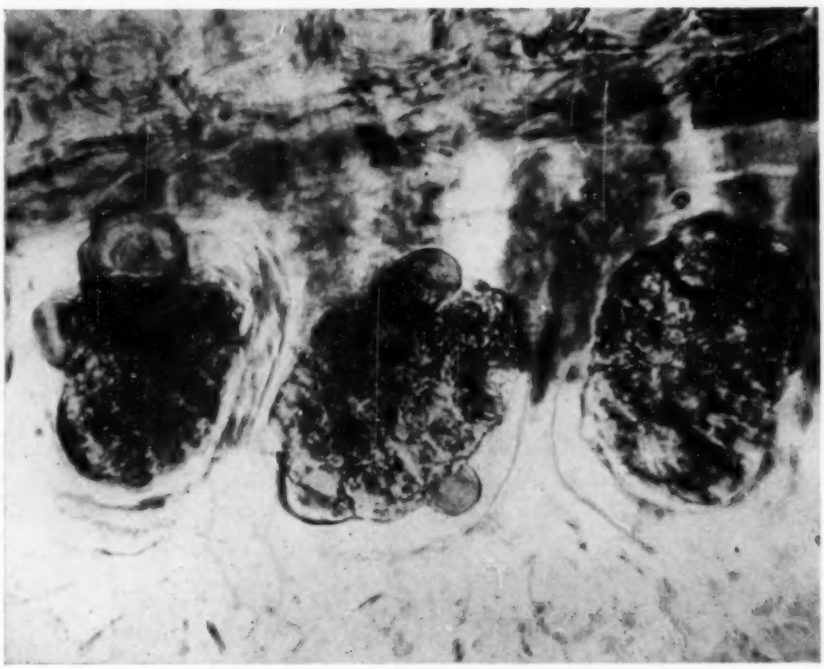


Fig. 1. Aleurone layer of bran stained with Sudan IV showing droplets of oil.

An examination of sections of the wheat grain was also made and it was found that the aleurone layer shows the presence of fat globules as was the case in unextracted bran. No fat could be discovered in the endosperm, while in the embryo, the plumule (shoot) showed very faint traces of fat and this appeared to increase in amount toward the primary and secondary roots, but the root caps were free of fat. The scutellum was heavily charged with fat, as well as the epithelial layer, which abuts upon the endosperm. The fat in embryo, scutellum and epithelial cells was found to be evenly distributed through the cell protoplasm and globules of fat were never discovered. These globules of lipide material appear to be entirely restricted to the aleurone layer and do not seem to occur in other parts of the wheat grain.

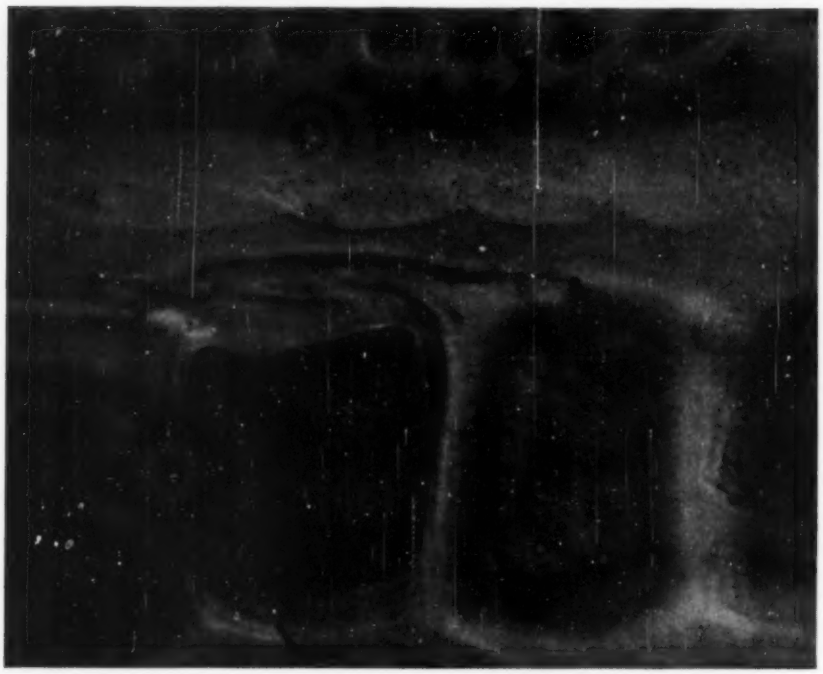


Fig. 2. Aleurone layer of bran stained with Sudan IV after extraction with petrol ether; no oil droplets left.

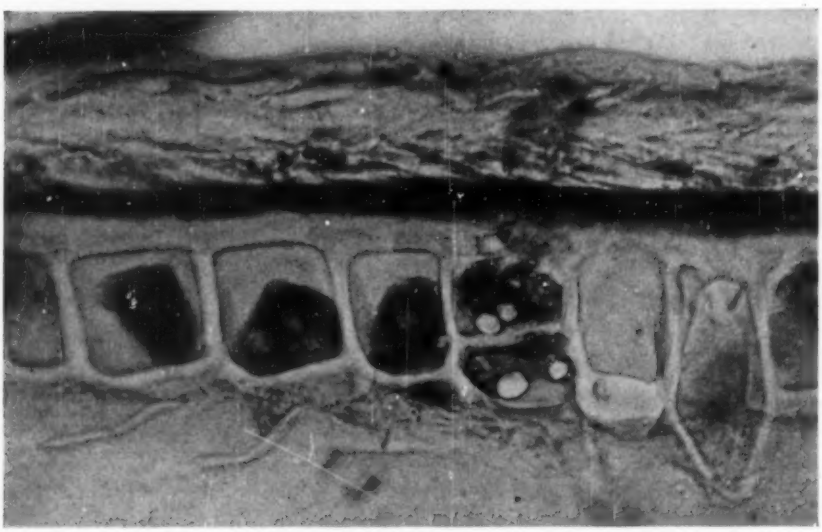


Fig. 3. Aleurone layer of bran stained with Sudan IV after extraction with benzene-alcohol mixture. Note testa is still deeply stained indicating presence of oil.

The persistence of fat in the testa is curious. This layer must presumably be charged with true fats, *i.e.*, glycerides of fatty acids or free fatty acids, since both Sudan III and Sudan IV only stain fatty acids and their glycerides, and do not stain phosphatides, cerebrosides or sterols. It is difficult to understand how the fat in this layer is able to withstand extraction first with petrol ether and then with a benzene-alcohol mixture for periods of 36 to 48 hours.

Summary

A chemical examination has been made of the crude oils extracted by petrol ether from bran, germ, bottom 1½%, bottom 12½% and patent flours from the same mill feed, all taken off the mill at the same time. It was found that the usual chemical constants, *i.e.*, acid value, saponification value, percentage of saturated and unsaturated fatty acids, molecular weights of these acids and their iodine values showed no significant differences, each value being substantially the same for all five stocks examined. The iodine values of the oils, however, differed significantly and showed a progressive decrease from bran and germ oil to patent flour oil. The unsaponifiable residue from these oils showed no significant difference in amount (4 to 5%) in any of the fractions, with the exception of bran oil (unsaponifiable residue = 12.1%). The unsaponifiable residue of bran oil also differed in physical properties from that isolated from the other milling fractions. In all the other cases the unsaponifiable residue was found to consist of a yellow oily fraction and characteristic feathery crystals, whereas in bran oil the unsaponifiable residue was composed of a dark waxy solid and feathery crystals.

The compound lipide fractions, *i.e.*, the fractions precipitated by the addition of acetone to the petrol ether extracts, were found in germ oil and in low-grade and patent flour oils to consist mainly of the magnesium salt of the nitrogen-free lipide, phosphatidic acid, together with small amounts of lecithin and kephalin. In bran oil, on the other hand, the compound lipide fraction was composed mainly of lecithin and kephalin; only a small amount of the magnesium salt of phosphatidic acid was present together with over 1.6% of iron the form of combination of which has not yet been determined.

A second lipide fraction was obtained from each of the various milling fractions, after preliminary extraction with petrol ether, by further extraction with a mixture of 80 parts of benzene and 20 parts of alcohol. The amount of the second extract was found to vary with the nature of the milling fraction. It was highest in bran and lowest in patent flour. Like the petrol ether extract the second lipide fraction

was found to contain simple lipides, *i.e.*, true fats, and compound lipides but a detailed examination has not yet been made.

Acknowledgments

The author desires to thank E. A. Fisher, Director of Research, for originally suggesting this problem for investigation and for his valuable suggestions and criticisms during its progress; also to thank F. Kidd and the Committee of Management of the Low Temperature Research Station, Cambridge, for according facilities for carrying out a great part of this investigation at Cambridge.

Literature Cited

Barton-Wright, E. C.
1938 Studies on the storage of wheaten flour. III. Changes in the flora and the fats and the influence of these changes on gluten character. Cereal Chem. **15**: 521–541.
Chibnall, A. C., and Channon, H. J.
1927 The ether-soluble substances of cabbage leaf cytoplasm. II. Calcium salts of glyceridephosphoric acids. Biochem. J. **21**: 233–246.
Jamieson, G. S.
1932 Vegetable Fats and Oils. The Chemical Catalog Co., New York.
Jordon, R. C., and Chibnall, A. C.
1933 Observations on the fat metabolism of leaves. II. Fats and phosphatide of the runner bean (*Phaseolus multiflorus*). Ann. Botany **47**: 163–186.
Kaufmann, H. P., and Keller, M.
1929 Determination of fats containing linolenic acid by the thiocyanogen method. Analysis of linseed oil. Z. Angew. Chem. **42**: 20, 73.
Kay, W. W., and Whitehead, R.
1935 The staining of fats with Sudan IV. J. Path. Bact. **41**: 303–305.
Rewald, B.
1936 The fat and phosphatide content of wheat germ. Chem. and Ind. **55**: 1002–1003.
Smith, J. A. B., and Chibnall, A. C.
1932 The glyceride fatty acids of forage grasses. I. Cocksfoot and perennial ryegrass. Biochem. J. **26**: 218–234.
1932a The phosphatides of forage grases. *Ibid.* **26**: 1345–1357.
Sullivan, B., and Bailey, C. H.
1936 The lipids of the wheat embryo. I. The fatty acids. J. Amer. Chem. Soc. **58**: 383–390.
1936a The lipids of the wheat embryo. II. The unsaponifiable fraction. *Ibid.* **58**: 390–393.

Corrections

In a previous paper by E. C. Barton-Wright (Studies on the Storage of Wheaten Flour—III, Changes in the Flora and the Fats and the Influence of these Changes on Gluten Character, Cereal Chem. **15**: 521–541) the following errors should be noted.

Page 524, lines 6 and 17, for *decrease* read *increase;* page 531, line 1, read *increase* instead of *decrease* and on the same page, line 6 *above* instead of *below*, line 8 *less* instead of *greater*, line 10 *higher* instead of *lower*, and line 12 *increase* instead of *decrease;* page 536, Table VI, for pH = 65.1 read pH = 6.51; page 530, lines 14, 16, and 19, read *pH* instead of *hydrogen-ion concentration.*

RELATIONSHIP OF MIXING SPEED TO DOUGH DEVELOPMENT [1]

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(Read at the Annual Meeting, May 1938)

For several years the baking industry has been offered so-called "high speed" mixers, without, however, having any definite standards by which to rate the relative degree of mixing action accorded the doughs per unit of time. Undoubtedly the term "high speed" has covered a fairly wide range of action, although all the machines so described are more vigorous and rapid in dough formation than the older types of single- or even double-arm, slow-speed mixers. It appeared desirable to investigate the optimum mixing treatment at different speeds of operation of the same mixing machine, and to this end a special mixer [3] was constructed and made available to us. The plan of operation was to estimate, for each of two different flour types, the work input necessary to produce optimum dough properties when using the mixer at various rates of speed.

Equipment

The mixer used for this study was a Hobart C-10 mixer to which a special pulley attachment had been added, making variable speeds obtainable. The McDuffee mixing bowl and arm were used throughout. The relative speeds referred to will be revolutions per minute of the pin holding the mixing arm. Speeds of 16, 40, 60, 80, 120, and 160 r.p.m. were used, representing a range of mixing action wider than that encountered in commerical mixers.

Small loaves were made in the regular A. A. C. C. pans. The doughs were fermented at 30° C. with pan proof at the same temperature. The bread was baked at 230° C. in an oven with a rotating shelf. The loaf volumes were determined with the Werner displacement apparatus by means of rape seed.

Materials Used

Two commercial flours were used, a strong flour (A) with 14.25% protein content at 15% moisture, and a medium-strength flour (B)

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[3] The authors gratefully acknowledge the cooperation of the American Dry Milk Institute in loaning a special variable-speed experimental mixer built especially for these investigations.

with 11.14% protein. These two flours thus represent types encountered in commercial practice, and moreover cover a fairly wide range of strength and composition. In view of the extensive use of milk in baking, doughs were prepared both with and without dry milk solids.[4] When the latter was included, it was uniformly in the proportion of 6 parts by weight to each 100 parts of flour. The dry milk solids used were of high quality and manufactured by the spray process.

Formula and Methods

The straight dough method was used throughout and the formula included 2% salt, 3% hydrogenated shortening, 5% sugar, 3% yeast, and in the milk doughs 6% dry milk solids. The absorption used for flour A was 69%, with milk solids 74%; for flour B 60%, with milk solids 65%.

Since the rate of development of a dough depends somewhat on the amount of dough in the mixing bowl, it was decided to mix 300 grams of flour doughs when 6% dry milk solids was used, and 319 grams of flour doughs without milk, thus making the total amount of dough approximately the same in all instances. The doughs were mixed at 30° C.

After mixing, the doughs were aliquoted into three 150-gram portions which were subjected to two, three, and four hours' fermentation, respectively, to study the relation of fermentation time to dough mixing and development. The two-hour doughs were punched after 1½ hours' fermentation and panned 30 minutes later. The three-hour doughs were punched after 1½ and 2½ hours, and the four-hour doughs were punched after 1½, 2½, and 3½ hours' fermentation. The pan proof in all cases was for 55 minutes at 30° C.; the loaves were then baked for 25 minutes at 230° C. The oven spring of the loaves was estimated by measuring the height at the center before and after baking, and the difference was recorded as "spring" in centimeters.

The loaf volume was measured after about two hours' cooling of the bread, and the loaves were assigned texture and grain scores the following day on the basis of 10 as superior. The formula used to compute the baking score, as a relative numerical expression of the bread quality, was as follows:

$$\text{Baking score} = 0.2\,(\text{LV}-400) + 2\text{T} + 4\text{G},$$

where LV = loaf volume in c.c.,
T = texture score, based on 10 as superior, and
G = grain score, based on 10 as superior.

[4] Dry milk solids is the product resulting from the removal of fat and water from milk. It contains not over 1½% butterfat and not over 5% moisture.

Variations of this formula have been used by other workers, but for this particular study the formula above was adopted because it placed relatively more weight on the grain score.

To study the effect of mixing speed, it is first necessary to ascertain the optimum mixing time at each speed and for each type of dough. For this purpose a series of baking tests were conducted at speeds of 16, 40, 80, 120, and 160 r.p.m. and with variable length of mixing time. For the slowest speed the mixing time was varied from 10 to 45 minutes and for the fastest speed from 1 to 5 minutes. From the overmixed or undermixed conditions of certain of these doughs and the resulting bread scores, the optimum mixing time was estimated. Four different types of dough, namely flour A and flour B each with and without dry milk solids, were mixed at the various speeds in order to establish the optimum combination of mixing speed and time, before any direct comparison of the effect of mixing speed could be carried out. Approximately 400 loaves of bread were baked and scored to establish the relationship between mixing time and mixing speed for optimum results. In estimating the optimum mixing time, consideration was given only to the two- and three-hour fermentation doughs which appeared to give nearly the same optimum. It was apparent that the four-hour doughs really required less mixing than the doughs fermented two or three hours. This is in agreement with other studies and shows that mixing and fermentation time tend to have an inversely related compensating effect.

To include the data for the 400 loaves of bread baked would require much space, and Figure 1 shows graphically the estimated optimum mixing-time curves for the four different dough types. Flour A required more mixing than flour B, and each flour with 6% dry milk solids required more mixing than without milk. The two extreme speeds were not used for the milk-free doughs and the dotted lines in Figure 1 are extrapolations of the experimentally determined values. Figure 1 shows that the relation between mixing time and mixing speed is not linear, which demonstrates that the number of turns of the mixing arm is not directly related to dough development. For instance, flour A with 6% dry milk solids required 688 revolutions of the mixing arm at 16 r.p.m. and only 560 revolutions at 160 r.p.m. As the speed is increased the mixing arm has a more vigorous action on the dough with each turn, and a pulling and stretching effect, probably changing with faster speeds to a tearing action.

The condition of the dough just after mixing is of interest. In the instance of flour A with 6% milk solids, for example, the doughs mixed 16 and 40 r.p.m. respectively were not sticky even on prolonged mixing. At 80 r.p.m. the doughs became sticky after 16 minutes' mixing, at 120

r.p.m. after 7 minutes, and at 160 r.p.m. after 2.5 minutes. Doughs mixed longer than this were extremely sticky when removed from the mixer. However, all these sticky doughs regained a normal condition within 90 minutes.

Figure 1 shows that the optimum mixing-time curves tend to converge as the speed is increased, thus making the optimum range more narrow and the effect of slight overmixing more critical.

Only the optimum mixing time has been determined thus far, and these results cannot be used for a critical estimation of preferred mixing speed, since the data for each speed were obtained on different days and the optimum time was sometimes interpolated between two

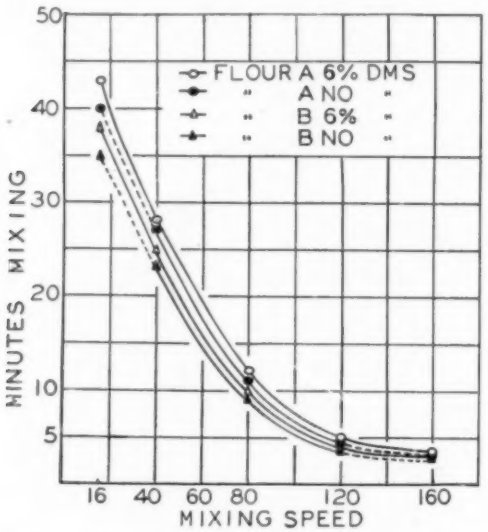

Fig. 1. Optimum mixing-time curves based on two and three hours of fermentation. Dotted lines show extrapolations.

actual observations. Accordingly, the next step was to mix doughs at the different speeds along each curve under identical conditions and carefully score the resulting bread. The resulting data can be compared graphically most conveniently by plotting the baking scores against the mixing speeds as in Figure 2. It is apparent that speeds of 60 and 80 r.p.m. almost invariably show slightly higher scores. Slower speeds than 60 r.p.m., that is, 40 and 16 r.p.m., show a rather rapid decrease in score, probably due to the time element involved in the long mixing periods. The decrease in score is not so pronounced with speeds higher than 80 r.p.m.

The improving effect of 6% dry milk solids is noticeable with both flours, but particularly with the medium-strength flour B. Both

flour A and flour B when used with 6% dry milk solids show greater tolerance to variation in mixing speed than the milk-free doughs, as shown by the more level character of the curves for the milk doughs.

Thus it appears that even if the optimum mixing time is used, the medium speeds of 60 and 80 give slightly better results. The first series of baking tests indicated that with the slower speeds the range of the optimum mixing time is quite wide, while with the faster speeds of 120 and 160 r.p.m., a minute or two of overmixing will prove quite disastrous, especially to weaker flours. Thus there is the added advantage in using medium speeds that a wider margin of optimum mixing time is assured.

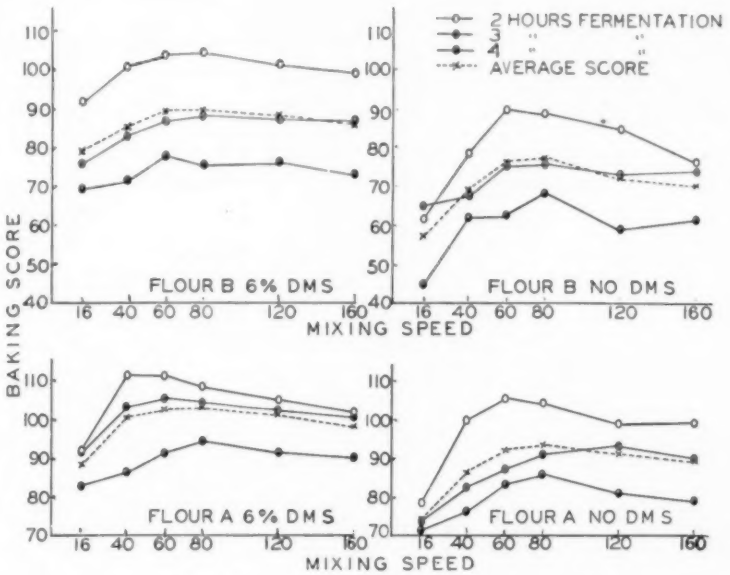

Fig. 2. Baking scores of bread made from doughs mixed for the optimum mixing times with various speeds.

It would be difficult to compute the speeds used into terms of the speeds of commercial mixers, but it seems that speeds of 60 and 80 produce a rate of dough development somewhere between that of the slow rye-dough mixer and the higher-speed commercial mixer. Further investigations of this nature conducted with commercial equipment would be desirable. Speeds of 60 and 80 r.p.m. can probably be referred to as medium high.

While it will require actual experimental observations to establish optimum speeds and length of mixing time for sponge doughs, it appears probable that these will require treatments essentially similar

to those which are optimum for straight doughs. Moreover, the work of Frey, Freilich, and Ekstedt (1937) indicates that sponge mixing is less critical than dough mixing when the sponge-dough method is used. They state that "sponges were mixed for varying periods of time without producing any apparent differences in the resulting bread," and later that "dough mixing proved to be a much more critical step in the procedure than sponge mixing or sponge fermentation." Their conclusions were based on both laboratory and bakery-sized doughs.

Our observations on oven spring indicate that underdeveloped doughs have less oven spring than those given proper mixing treatment. Overdevelopment also seems to lower the oven spring. The amount of oven spring is also affected by the duration of pan proof, which was 55 minutes at 30° C. for this work.

Plasticity Studies

Farinograms were recorded with flours A and B, without dry milk solids and with 6% of the latter. All ingredients (except for the yeast) were used as for the baking tests, including water. The same sized doughs of 300 grams of flour for milk doughs and 319 grams of flour for milk-free doughs were used in the farinograph as for the baking tests, thus again keeping the total amount of dough constant. The doughs were mixed for 30 minutes at 30° C. The farinograms show the differences in the rate of development and relative plasticity of the doughs.

An attempt was also made to study the plasticity of yeast-free doughs mixed in the McDuffee bowl with the variable-speed mixer, using the optimum mixing time for each speed. An effort was made to have the temperature of the doughs at 30–31° C. when mixed. Immediately after mixing, the doughs were transferred to the farinograph bowl and mixed for ten more minutes. From the farinograms produced, the extent of development of the doughs in the variable-speed mixer could be estimated. Figure 3 shows the ordinary farinograms, followed by the ten-minute curves of the transferred doughs. The interval A to B of the thirty-minute curves appears to be the part reproduced in most cases by the transferred doughs. Thus it is apparent that the doughs mixed along the optimum-time curves had all reached approximately the same stage of development insofar as such methods will disclose. This is more significant in view of the fact that the optimum-time curves were determined solely by the baking results. Certain of the ten-minute curves show a higher plasticity and steeper slope than the corresponding thirty-minute curve, which may be due to thermal change. This effect of a more rapid breakdown with lower

mobility is well recognized by those familiar with farinograms. Thus the differences in plasticity observed here are probably the result of temperature differences.

It is quite apparent that the same degree of development was reached by the dough regardless of the mixing speed, when the optimum time of mixing was allowed. Another significant feature of the farinograms is the time required to reach the point A, which apparently represents the point of proper development of the doughs. With the milk-free dough prepared with flour A the time was 14 minutes, and when the milk was used it was 16 minutes; for flour B, milk-free, it

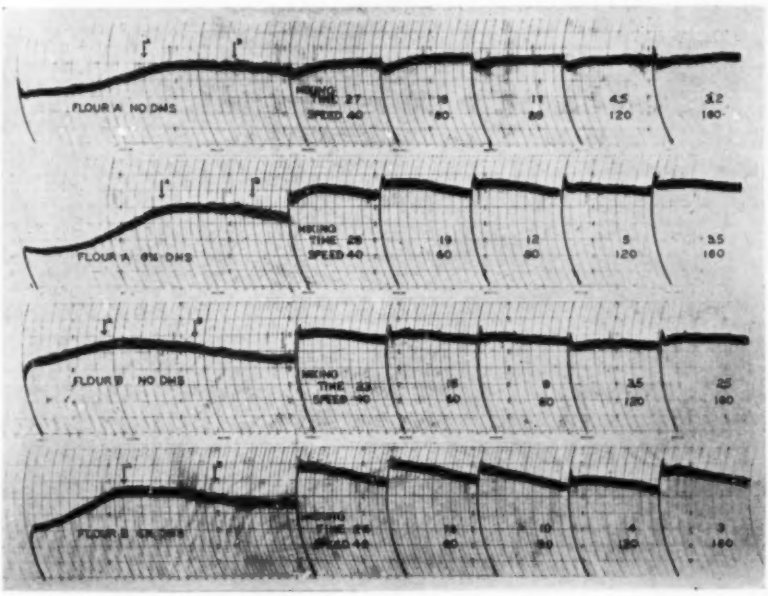

Fig. 3. Farinograms of doughs containing all ingredients except yeast. The thirty-minute curves to the left were from doughs mixed entirely in the farinograph. Ten-minute curves are of corresponding doughs mixed in the variable-speed mixer and then transferred to the farinograph. Interval *A* to *B* appears to be the part of the thirty-minute curve represented by the transferred doughs.

was 9 minutes, and with milk 11 minutes. The relative mixing periods for the four doughs are in the same order as shown by the optimum mixing-time curves in Figure 1. Bohn and Bailey (1936) observed that the point of maximum plasticity on the farinograms appears to be the optimum dough development as far as baking quality is concerned. This is in substantial agreement with these results, since point A on the farinograms is just before the point of maximum plasticity or minimum mobility. Combining these observations it appears that the mixing speed of the farinograph is equivalent to speeds of 60 to 80 r.p.m. of the other mixer. Hence the rate of dough development of the

farinograph appears to be close to the optimum. This is not entirely in accord with the observations of Bohn and Bailey (1936), who found that doughs mixed in the Hobart-Swanson mixer gave bread scoring slightly higher than from doughs mixed in the farinograph. The higher scores were due mainly to larger loaf volumes, and the grain and texture scores were about the same. They fermented the doughs for only one time period, three hours. They further noted that "it is claimed that high-speed mixers make it possible to incorporate slightly larger amounts of water into the dough than low-speed mixers. The increase does not appear to be very significant, providing each dough is mixed to approximately the same stage of development."

Determination of Work Input in Watt Hours for the Various Doughs

The use of a watt hour meter to study the work input of various doughs was described by Bailey (1930) who found the amount of work input varied with the water added per unit of flour. St. John and Bailey (1929) used the same method and found that the water-imbibing

TABLE I

WORK INPUT, IN WATT HOURS, OF DOUGHS MIXED FOR OPTIMUM TIME AT EACH SPEED

Mixing speed	Flour A, 6% D.M.S.		Flour A, no D.M.S.		Flour B, 6% D.M.S.		Flour B, no D.M.S.	
	Mixing time	Work input	Mixing time	Work input	Mixing time	Work input	Mixing time	Work input
R.p.m.	*Min.*	*Watt hrs.*	*Min.*	*Watt hrs.*	*Min.*	*Watt hrs.*	*Min.*	*Watt hrs.*
40	28	11.79	27	8.59	25	7.76	23	6.33
60	19	11.39	18	8.29	16	7.89	15	6.99
80	12	10.32	11	7.79	10	7.23	9	5.46
120	5	10.92	4.5	8.69	4	6.83	3.5	4.86
160	3.5	10.09	3.2	7.33	3	6.96	2.5	5.29
Av. watt hour		10.88	——	8.14	——	7.55	——	5.79

capacity of a dough increased with the addition of dry skimmilk at the rate of about a unit weight of water for each unit of dry skimmilk. We employed a similar method to determine the work input in watt hours in mixing doughs along the optimum-time curves. The resulting data are recorded in Table I and represent values obtained in mixing the doughs represented in Figure 2, except that values for a mixing speed of 16 r.p.m. are not included.

The data in Table I indicate that, regardless of the speed, during optimum mixing time the work input was about the same for each treatment with any single dough formula. The values fall into four distinct groups in the same relative order as the optimum mixing-time

curves in Figure 2 and the optimum period of development as shown by the farinograms in Figure 3. Thus it appears that the amount of work input required for proper development of a particular dough is about the same regardless of the mixing speed and rate of development.

Summary

To study the effect of mixing speeds on dough development, the straight dough method with 2, 3, and 4 hours of fermentation was used. Small loaves, each 150 grams of dough, were made throughout. A strong flour and a medium-strength flour, without milk and with 6% high-quality dry milk solids, were used.

For each speed of rotation of the mixing blade an optimum mixing time was found. This time was longer for a strong flour than for a medium strong. Doughs with 6% dry milk solids required longer mixing time than milk-free doughs. Thus the inclusion of dry milk solids has the same effect as using a stronger flour. This was especially evident with the medium-strength flour.

Bread scores from doughs mixed along the optimum-time curves were slightly higher at the medium speeds of 60 and 80 r.p.m. Slower speeds gave a rapid lowering in score, while speeds faster than 60 to 80 r.p.m. resulted in a decrease in score, but the rate of decrease in score was more rapid in the low-speed range. When the optimum time of mixing was allowed, doughs with 6% dry milk solids showed a greater tolerance to variation in mixing speed than when the milk solids were omitted.

Oven spring of most doughs increased with increased mixing and development until a highly overmixed condition was reached, when the oven spring began to decrease.

Farinograms of doughs previously mixed in the variable-speed mixer indicated that the doughs mixed along the optimum time curves had reached approximately the same stage of development in each instance.

The point just before the peak of maximum plasticity on the farinograms appeared to be the stage of optimum development of a dough as far as baking quality was concerned.

A study of work input in mixing doughs, as measured by a watt hour meter, showed that when mixed along the optimum-time curves the work input was nearly the same, regardless of mixing speed. Strong-flour doughs required more work for optimum development than doughs prepared with flours of medium strength. Doughs made with flour plus 6% of dry milk solids required more work in mixing than when the milk solids were omitted.

A medium to moderately high mixing speed resulted in superior bread. With reduced speeds the bread was inferior even when the optimum time of mixing for that speed was allowed. This may be the consequence of the relatively long time requisite for dough development. High mixing speeds resulted in satisfactory bread when the mixing time was carefully adjusted, but the bread scores were higher when medium mixing speeds were employed. Another advantage in favor of the medium speeds is that the range of time through which mixing can be continued for optimum results is much less narrow than when higher speeds are involved. This is particularly significant in the instance of weaker flours which may easily be overmixed at the higher speeds.

Literature Cited

Bailey, C. H.
1930 A method for measuring the relative plasticity of pastes and doughs. Rheology **1**: 429–432.

Bohn, L. J., and Bailey, C. H.
1936 Effect of mixing on the physical properties of doughs. Cereal Chem. **13**: 560–575.

Frey, Charles N., Freilich, Jacob, and Ekstedt, Harry.
1937 Correlation of experimental and commercial baking tests when using sponge doughs, II. Cereal Chem. **14**: 629.

St. John, J. L., and Bailey, C. H.
1929 The effect of dry skimmilk upon the water absorption of doughs and the plasticity of flour suspensions. Cereal Chem. **6**: 140–150.

PARTICLE SIZE IN RELATION TO FLOUR CHARACTERISTICS AND STARCH CELLS OF WHEAT

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(Received for publication June 18, 1938)

The question of granulation, *i.e.*, the influence of the size of particles on the characteristics of flour, has been dealt with in many publications. It constitutes a milling problem of major interest and importance. Investigations have been chiefly along two different lines. Some workers have divided flours into various groups according to the size of the particles and then investigated the behavior of each group as tested by different chemical and other methods. Among these publications the following may be mentioned: Le Clerc, Wessling, Bailey, and Gordon (1919) and Kress (1929). Other investigators have reduced the same material to various degrees of fineness and have then examined the qualities of the flours. From this group the following papers may be mentioned: Shollenberger, Marshall, and Hayes (1921) and Alsberg and Griffing (1925). Shollenberger and Coleman (1926) have worked on both questions.

It must be pointed out that although the investigations of the former group may seem to be—at least at first glance—markedly more important to the practical milling and baking trades, the latter method, however, offers better possibilities for explaining the fundamental question of the effect of particle size. Since the compositions of the samples are at least very nearly the same, the differences must depend either entirely or almost entirely on the variations in the size of particles. Nevertheless it has been regarded necessary to make, in some investigations of the latter group, certain analyses of a purely chemical nature (ash, Kjeldahl-N, etc.). The differences obtained are naturally slight, sometimes being within the range of error of the analytical methods. On the other hand, more pronounced and interesting results have been obtained by investigating enzymatic phenomena (such as increase in amylolytic activity), or by employing other methods which are better adapted to flour testing than are the ordinary means of the analytical chemist.

It is a common feature in all these studies that no attempt has been made to avoid secondary phenomena—due to the severe and

rapid grinding of flour—such as heat development, which may cause some slight chemical, especially enzymic, changes. On the contrary, the grinding has usually been made purposely more severe in order to increase the effect of the secondary phenomena. Therefore the investigations cited above yield valuable information on the influence of "excessive grinding" or "overgrinding."

The present study belongs in the second group of investigations, differing, however, from these particularly in that it is confined strictly to the effect of the particle size. For this reason the grinding was carried out with extreme care in order to prevent any possible heat development. Therefore no marked chemical changes could be expected and chemical analyses were regarded as unnecessary. It is not very likely that a slight contact with a cool steel surface would cause chemical changes that could be detected by generally known analytical methods. Nevertheless, the behavior of the flour was in some respects substantially altered by grinding. Thus for instance the saccharogenic activity is enormously increased as the size of the particles becomes smaller, although it can hardly be expected, nor is it even necessary to assume, either that starch would undergo any changes of a chemical nature or that the amounts of the amylolytic group of enzymes would be increased by the mechanical treatment.

A further reason why the writer has desired to investigate this question is that there are at present some new methods available which were not known at the time the previous reports were published. It is very interesting, for instance, to compare the results obtained by different methods and to investigate the extent to which the results of such very objective measuring methods as the farinographic and fermentographic tests agree with those obtained by experimental baking and some other methods. Furthermore, it must be stated that while in many of the previous reports a wealth of information concerning very fine and medium-fine flours is obtainable, the coarser products have been rather neglected. It was therefore thought desirable to obtain additional knowledge of the baking value of the coarser products, especially in view of the fact that these types (the so-called "granular flours") are of considerable importance in certain countries. In addition to these questions, special attention has been paid to the microscopic structure of starch cells and the changes which they undergo as a result of mechanical treatment.

Materials and Methods

A mixture of fine semolina and middlings, as experimental material, was taken from a commercial mill, not only because the purifier problem was difficult to handle under laboratory conditions but also

because of the great amount of material needed. Moreover the original wheat mixture had been carefully studied and found to have satisfactory baking qualities. The mixture consisted of 44% Manitoba (Nos. 1–4), 20% Hungarian wheats (of medium strength), and 36% of Finnish varieties (medium-strong and rather weak). The general baking properties of the mixture are shown clearly enough by the data that are to follow.

In order that the test material would closely correspond to the original wheat mixture and to the resultant commercial flours whose properties were known from experience, the material was taken from a comparatively wide range of purifier sieves. The material obviously included many different sizes of particles. The fact is not however of much importance since it would not have been possible to improve the homogeneity in the later stages of reduction if it was desired (as

TABLE I

Results Obtained in Mechanical Sieving Tests

Test No.	Material, ground to pass through	Average length of aperture, μ	Percentages of material passing through the following sieve numbers				
			60 g.g.	6 xx	10 xx	14 xx	25 xx
0	(Original material)	—	54.6	19.1	2.5	1.8	0.8
1	60 g.g.	300	—	29.3	6.2	4.3	1.9
2	6 xx	200	—	—	17.0	9.9	3.5
3	10 xx	125	—	—	—	72.0	24.5
4	14 xx	88	—	—	—	—	40.5
5	25 xx	57	—	—	—	—	—

already mentioned) to keep them identical in chemical composition. The original material had passed through purifier sieves ranging from 36 g.g. to 64 g.g. The results obtained in sieving tests (Table I) will indicate the particle sizes of the original materials, as well as the subsequent degrees of fineness.

Table I includes also the averages of the measurements the writer has made on the mesh apertures of the various silks. The opening was considered as a regular parallelogram. The measurements were made under a microscope with Skar's ocular micrometer. The relative atmospheric humidity was approximately 40% and the temperature about 20° C. The results are in good accordance with those presented by Haltmeier (1928). As can be seen from Table I, the cloths employed in the sieving tests were selected so that the linear increase of the mesh aperture from any sieve to the next one was approximately

50% and consequently the simultaneous areal increase was more than double.

It is of course clear that the actual size of the flour particles cannot be exactly determined by simple sieving analyses (see for example Markley, 1934). The method was chosen in spite of its inexactness because there were no other reliable and practical methods available. Besides, this method gives at least the *maximum* particle size at each stage of reduction. This is true also in regard to the last degree of fineness, as will be shown later. Furthermore the regular increase in the percentages given in Table I is likely to suggest that a certain amount of reliable information can be obtained as to the quantities of different-sized particles contained in each group. Such information was regarded sufficient, and a very exact knowledge of the actual sizes of particles would hardly be of value, since each group was, as already pointed out, somewhat heterogeneous.

The use of sieving tests in the determination of the real sizes of the particles becomes the more uncertain, the smaller the particles. If moreover the material is very dry, the difficulties are increased. This last-mentioned observation in conjunction with certain other facts has induced the writer to assume that electrical phenomena can play an important and in some cases even decisive role in the bolting process. (*Cf.* also Van der Lee, 1928.) Some experiments to ascertain the significance of electric forces in the bolting of flour have already yielded support for the assumption advanced. The work is still in progress, but will be finished in the near future.

The raw material obtained from the big mill (altogether about 30 kg.) was mixed thoroughly in a special rotating container. The mixture was kept in a tightly closed sheet-iron box until the required portion was taken therefrom for each grinding test. In this way it was fully ascertained that the raw material was exactly the same in each test. The moisture of the material was 16.6%.

The reductions were carried out in a small laboratory experimental mill (Seck) and a separate miniature plansifter (Miag). The rolls are 120 mm. long and 80 mm. in diameter. The speeds of the (smooth) rolls were 377 and 302 r.p.m. Consequently, the highest speed of the roll surface was nearly 1.6 meters per second, the lowest approximately 1.3 meters, and the difference about 0.3 meter per second. In grinding, the lowest possible roll pressures were used. This was facilitated by a specially arranged scale attached to the roller mill. Since very moderate feeds were used and the grinding was carried out in a cool room, the surfaces of the rolls were kept perfectly cold. A fact of greatest consequence may however have been the removal of the

material which had reached the required fineness, after every passage. Thus the unnecessary wearing down of the material was reduced to a minimum. The grinding process was repeated until all the material was put through the given mesh number. In the case of the finest cloth (No. 25 xx), it was necessary to grind a very small amount of fibrous material in a porcelain mortar in order to get it sufficiently fine.

The method of grinding employed in these experiments is of course very slow and laborious, and has not much in common with the grinding in a commercial mill, where fairly abundant feeding and particularly higher pressures of the fast-running rolls cause a considerable warming up of the rolls and the wheat material. Considering the nature of the grinding method it was justifiable to assume that the differences observed in the products reduced to various degrees of fineness were ascribable, at least for the most part, to the different particle sizes and not to any secondary chemical phenomena caused by rapid and severe grinding.

Investigation of the Products

Particular attention was paid to methods that would be, as much as possible, free from personal judgment. Therefore the baking tests were supplemented by the entirely mechanical farinographic method, as well as by Berliner and Koopmann's gluten-testing procedure, which is easy to perform and is decidedly objective. Amylolytic (diastatic) activity was examined by means of a Brabender fermentograph and the Blish-Sandstedt maltose test. In addition to these methods generally employed in cereal chemistry some special microscopic investigations on starch cells were carried out in order to find a plausible explanation of the great changes which occur in the amylolytic properties of wheat material as a result of grinding and, generally, to contribute to the present information on the microscopical structure of starch cells.

Gluten Testing and Farinograms

The farinographic method is generally considered to be very suitable for the determination of baking properties of flour in cases where flours, derived from different wheat mixtures but prepared by fairly similar grinding methods, are to be compared. It was indeed of interest to find out what kind of results this, as a known, applicable method, would yield under conditions where the chemical composition of different products was identical but the degree of fineness varied within wider limits than are usually found in milling and baking practice.

The farinograms are shown in Figure 1, and the water absorption

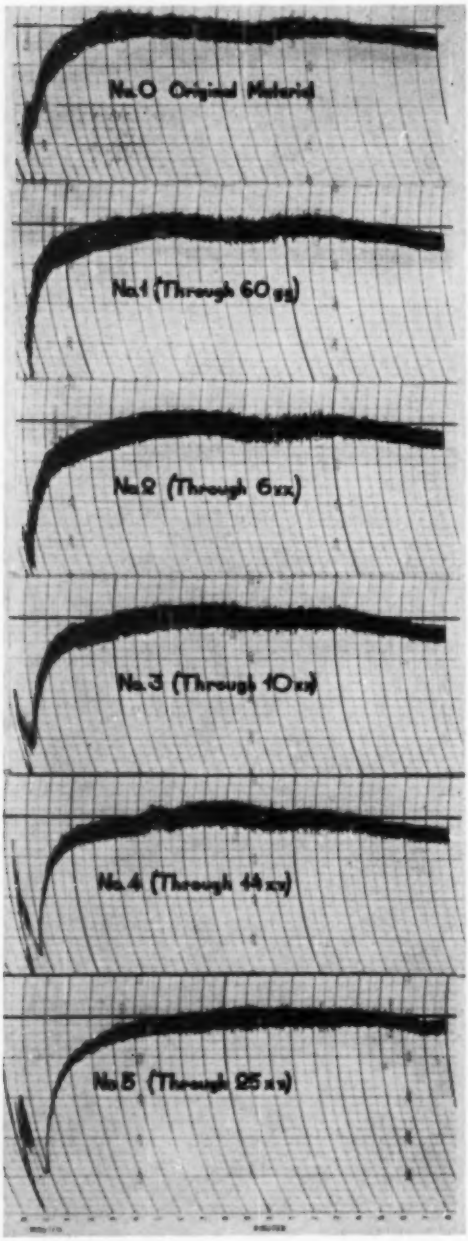

Fig. 1. Standard farinograms of materials reduced to different degrees of fineness.

and time of dough development measured by the farinograph in Table II. It is to be noted that while the absorption increases substantially with the decreasing size of the particles, the farinograms differ from each other only slightly. Thus farinogram No. 5 (through 25 xx) is apparently a bit narrower than average but its degree of weakening is nearly the same as that of others, although the baking tests showed No. 5 to be decidedly inferior. Certainly the deterioration could be regarded as due to excessive amylolytic activity (Table IV). Since, however, the quality of gluten washed free from starch has been altered very much, being abnormally hard in the case of No. 5, greater changes would reasonably be expected in the farinograms. Likewise the best result was obtained from No. 3 (through 10 xx) by all baking tests and with the method of gluten testing by Berliner and Koopmann, but the farinogram No. 3 cannot be claimed to be distinctly better than the preceding ones. It would moreover be most difficult to predict on the basis of the farinograms of Nos. 0 and 1 that the materials in question, which are indisputably too coarse, would yield as poor results as those obtained from the baking tests. This observation must be borne in mind whenever it is desired to estimate, solely on the basis of farinograms, the baking value of very coarse products, *e.g.*, such as are still used in certain countries under the name of "granular flours." All in all it seems that the farinographic method is not so well adapted to the treatment of the special problem dealt with in this study as it is to the investigation of flour of ordinary granulation.

The greatest variations are to be found in the phenomena connected with the absorption of water. The amounts of water absorbed by the different materials forming doughs of equal consistency (500 farinograph units) are considerably increased by grinding (Table II). The times of dough development, *i.e.*, the periods the farinograms need to reach their highest point and full width, are simultaneously lengthened (Fig. 1 and Table II). The increasing of these periods is especially very rapid in the beginning (compare Nos. 0 and 1). Thus, a finer flour forms dough more slowly than a coarse one, which is contrary to the commonly prevailing belief. Whether the retarding effect of fine grinding on the dough development is due to an actual decrease in the rate of absorption cannot be decided on the basis of this series of experiments. Divergent opinions as to this point are easily formed.

Table II presents the results of gluten tests made according to Berliner and Koopmann (patented method). In the amounts of wet gluten no distinct tendency in any direction is to be noted. The

TABLE II

EFFECT OF GRANULATION ON DIFFERENT PROPERTIES OF FLOUR

Material		Wet gluten (15% basis)	Characteristics of gluten, free from starch						Farinogram (consistency 500)	
			Swelling test of Berliner				Hand tests			
			Swelling figures			Clearness of liquid	Hardness	Distensibility	Water absorption (15% basis)	Development time
No.	Ground to pass through	%	*1 hr.*	*2 hrs.*	*2½ hrs.*				%	*Min.*
0	(Original material)	33.4	7.5	14.0	15.4	Slightly turbid	Soft	High	57.7	6.0
1	60 g.g.	36.0	7.5	13.5	15.8	Slightly turbid	Soft	High	57.9	8.0
2	6 xx	34.5	8.3	13.8	16.0	Slightly turbid	Normal	Medium	58.3	8.5
3	10 xx	34.0	8.8	15.5	17.0	Slightly turbid	Normal	Medium	62.0	9.5
4	14 xx	32.8	7.0	13.3	15.0	Clear	Hard	Low	65.7	10.0
5	25 xx	33.5	7.8	13.5	14.5	Clear	Very hard	Very low	71.8	11.0

differences in swelling figures are also small, but a discernible optimum is, however, at No. 3 (through 10 xx), which agrees well with the conception derived from baking tests. The figures presented in the table are averages from four series of tests made by two persons. Because all of the differences in swelling figures are slight, those for 1 hour and 2 hours have been included. What was said of the farinograms applies also to the swelling figures; that is, differences are not so great as could be expected. Particularly from the swelling figure of No. 5 it could not in the least be concluded that the flour in question was as inferior as was disclosed by baking tests. By hand manipulation, however, it was easy to notice that the gluten No. 5 was abnormally hard and short. Thus a careful reduction of the particle size, whereby no increase of temperature occurs, has, when carried sufficiently far, markedly injured the gluten properties. This is not difficult to understand, bearing in mind that the free openings of the finest sieve (No. 25 xx) are so small (Table I) that the biggest starch cells may pass the silk only one by one, while the smaller ones may pass in very small groups. As the gluten in its original state in the wheat grain is to a certain extent a coherent structure, it is not at all surprising that when torn into tiny particles it is not able to reform the very firm films that are necessary for an effective retention of gas. Nevertheless it is quite possible that the gluten washed free from starch would be firm enough to produce normal, or nearly normal, swelling figures.

Baking Tests

In test baking, the A. A. C. C. basic procedure and different modifications were employed in order to obtain the best possible results from each product under investigation. For instance, a series of experiments were carried out where amounts of water and periods of fermentation varied. As the products differed widely from each other, especially in their gas-production abilities, several experimental series were run, with additions of varying amounts of different diastase preparations (Merck, B. D. H., etc.) to the coarser products. Likewise in some experiments both the time of fermentation and diastase additions were simultaneously varied.

Table III shows volume results of some of the numerous baking tests. The greatest volumes were derived from those tests where the fermentation periods were varied. All tests led very consistently to

TABLE III

AVERAGE LOAF VOLUMES OBTAINED IN BAKING TESTS

Material No.	0	1	2	3	4	5
Ground to pass through	(Orig.)	60 g.g.	6 xx	10 xx	14 xx	25 xx
Method of baking employed	Loaf volumes per 100 g. flour, in c.c.					
A. A. C. C. basic procedure	330	390	420	475	420	345
With different amounts of water	355	395	425	445	440	375
With diastase addition (Diast. prep. added per 100 g.) *mg.*	460 (150)	460 (100)	470 (50)	475 (0)	435 (0)	385 (0)
Optimum loaf volumes with varying fermentation times and diastase additions	540 (200)	520 (100)	585 (50)	630 (0)	550 (0)	510 (0)

the result that the flour No. 3 (through 10 xx) had the best baking characteristics, whereas the results became worse for the coarser or the finer particle sizes. The results were in good accordance with the gluten properties as well as with the farinographic tests so far as definite conclusions from the latter could be drawn. As already pointed out, the differences obtained by baking tests are, however, greater than actually could be expected on the basis of the other experiments.

It is noteworthy that the loaf volumes of coarser products, in which gluten and starch are comparatively uninjured, can be considerably increased by diastase addition. However the finer flours, particularly No. 5 (through 25 xx), remained obviously the lowest in quality.

It may be mentioned as a special feature that the color of crumb was distinctly deteriorated in the too-fine and too-coarse flours (Nos.

0 and 5), both of which had a distinctly grayish tinge. The phenomenon has been stated previously to be characteristic of excessively ground flours (Alsberg and Griffing, 1925). The cause of the phenomenon becomes difficult to explain from the observation presented here. Since a loaf baked from exceptionally coarse flour has the same crumb color as that from very fine flour, the inferiority cannot apparently be due to excessive activity of amylolytic enzymes, as could be explained on the basis of previous observations. This applies, for the same reason, to other enzymes, *e.g.* tyrosinase (*cf.* Kozmin), which are occasionally mentioned in connection with the color of crumb.

Aside from the loaf volume and the color of crumb, the quality of the crust was also distinctly poorer in the loaves made from the coarsest flours, as well as in those made from the finest ones.

Amylolytic Activity

If it is desired to determine the amylolytic (diastatic) activity of a given flour, *i.e.* the measure of the enzymic function which results in decrease of starch and increase of reducing sugars and carbon dioxide, any of the numerous and practical methods now available may be chosen. Although any of these methods can give a sufficiently clear conception of the ability of the material in question to form carbon dioxide, they differ considerably, however, from each other. This is shown by the results here presented.

In the present work two well-known methods were employed simultaneously, the Blish-Sandstedt maltose method and the Brabender fermentograph. The results are shown in Table IV.

TABLE IV

EFFECT OF GRANULATION ON AMYLOLYTIC ACTIVITY

Material		Maltose after 1 hr. at 30° C. (dry basis)	CO_2 produced in fermentograph during successive hours of fermentation, fermentograph units (cc.)									
No.	Ground to pass through		*1 hr.*	*2 hrs.*	*3 hrs.*	*4 hrs.*	*5 hrs.*	*6 hrs.*	*7 hrs.*	*8 hrs.*	*9 hrs.*	*10 hrs.*
0	(Original material)	% 0.97	295	380	320	115	—	—	—	—	—	—
1	60 g.-g.	1.02	280	405	325	120	—	—	—	—	—	—
2	6 xx	1.22	280	380	390	120	100	—	—	—	—	—
3	10 xx	1.82	250	385	555	420	170	—	—	—	—	—
4	14 xx	2.67	235	400	575	620	345	—	—	—	—	—
5	25 xx	4.07	230	440	610	680	615	450	255	190	—	—
E	25 xx (overground)	5.93	200	435	580	615	540	470	400	360	330	300

Enormous differences are noted in the maltose values of the flours ground to various degrees of fineness, particularly from No. 2 upwards.

The mere reduction of the size of particles has, in the extreme case, increased the amount of maltose found in the suspension after one hour's fermentation at 30° C. to four times the original value (compare Nos. 0 and 5).

As it was of interest to note how much the maltose figures would rise if the grinding were performed with higher roll pressures, a special test was run. In this experiment all the material was finally passed through a 25 xx sieve, but at first the whole amount of flour was passed 20 times between very closely set rolls without separating the sufficiently fine flours between the passages. A slight warming up of the rolls was noticed. The resulting data are included in Table IV (material *E*).

As can be seen from the table, the maltose figure has become six times the original (Karacsonyi and Bailey, 1930). No exceptionally great difference is, however, noted between the fineness of No. 5 and of *E*. This fact shows that the increase of the maltose value has appeared as a consequence of the advanced degree of grinding and the method of grinding does not seem to have had any decisive effect upon it. Certainly the grinding could be accomplished still more vigorously, especially with large and fast-running rolls, whereby the heat evolution could probably cause some more noticeable changes in the material. On the other hand, the above suggestion applies only to the usual mode of grinding, *i.e.*, treatment with rotating rolls at a relative low moisture content. If some entirely different method were used, it would be at least theoretically possible that a high degree of fineness could be reached without effecting any great changes in the amylolytic activity of the material. This will be understood from observations presented farther on which make it very probable that the increase in the amylolytic activity is not directly connected with the size of flour particles, but is chiefly dependent on certain strictly definable mechanical changes in starch cells.

The results of fermentograph tests are presented in table form in order to save space (Table IV). It is interesting to note that the increase of amylolytic activity resulting from the reduction of the size of flour particles begins to effect significantly the amount of carbon dioxide only during the second hour, and even then very slightly. Not until later do the differences become marked. Thus for instance the amount of gas for the fourth hour in No. 5 (through 25 xx) is more than six times greater than in the original material (0). Comparing all the fermentographic figures in the table, it will be noted that with increasing fineness the most vigorous period of gas production is continually moving to later and later hours of fermentation. Most

surprising is the fact that the amount of gas for the first hour is smaller in finer materials than in coarser ones.

The above results show that although both of the methods employed here have yielded a similar picture of the amylolytic activity of the products in question, yet great differences exist between the methods. For this reason the adaptabilities of both methods may in certain cases be very different (Pulkki, 1938). As to the results obtained with the fermentograph, the values for the third and fourth hours seem to correspond best to the results of the Blish-Sandstedt maltose tests. It should be pointed out that these are the hours to be considered the most important in the fermentation of flours of the type of baking strength investigated here.

Starch Cells

The substantial increases in the amounts of maltose and carbon dioxide formed in the dough, as illustrated by Table IV, were caused solely by a careful mechanical treatment of the wheat material. As it cannot reasonably be supposed that such a treatment will increase the amount of enzyme, the cause of the increased enzymatic activity must be sought in some alterations in the materials governing the susceptibility of starch to the action of enzymes, for example in some mechanical changes which take place in the starch cells. This question has already been dealt with in some of the investigations referred to in the introduction. Thus Alsberg and Griffing (1925) report: "Overgrinding flour injures the starch granules so that a part of the starch swells and disperses when the flour is doughed. . . . One result is an increase of the cold water extract from dispersed starch." Shollenberger and Coleman (1926) have also confirmed this observation, although the differences found by them were smaller than by the first-mentioned authors.

Both the above reports and many other papers present the view that the starch granules are in some way injured during grinding, especially if the grinding is carried very far. Except for the increase of quantity of extract and the capacity of swelling, already referred to, the characteristics of the "injuries" to the cells caused by "excessive grinding" or "overgrinding" are not known in detail. It is however known that, because of grinding, an increased number of starch cells are stainable by Congo red, whereas the completely uninjured cells remain uncolored.

The present work has not emphasized the effects of "overgrinding"; on the contrary, high roll pressures and the secondary phenomena —particularly heat development—which are usually involved in

vigorous grinding, have been carefully avoided. Yet the reduction of the size of the particles has been carried very far.

Since the starch cells of wheat are approximately 30 to 40 μ in diameter, the largest ones being about 50 μ, the large and medium-sized cells can pass the finest silk employed in these experiments only one at a time, the average size of the openings being about 57 $\times$ 57 μ (see Table I). It is therefore justifiable to start with the assumption that the increased amylolytic activity is due to the fact that an increasing number of starch cells are broken, whereby the inner parts of the cells are exposed to the enzymic activity. In microscopic examination it was found that many of the starch cells were indeed split in two, and others had obtained cracks or fractures of some description. Therefore the direct breaking of cells was first considered as a plausible explanation of the multiplicate increases in the maltose and CO_2. That such was not, however, the case became evident from some simple experiments consisting of direct counting of differently changed cells under the microscope. The results of these experiments are presented below.

To facilitate the microscopic examination, a method of staining the cells was developed. At first a dilute iodine solution was used, but later another method, consisting of simultaneous use of iodine and Congo red, was found to be more practical.

Starch was washed from 10 g. of material to be investigated. The starch was suspended in water and filtered through a fine sieve (silk 25 xx). Nearly one quarter of the suspension was diluted to 200 ml. About 0.2 ml. of 10% alcoholic iodine solution was added to the suspension and, after 15 to 30 minutes, 3 to 5 ml. of 1% aqueous solution of Congo red. To obtain the optimum effect, the quantities of the staining reagents were adjusted in each case by experimenting.

In the microscopic measuring and counting of the cells an ocular micrometer (Skar) and a graduated object chamber (Bürker, Zeiss) were used.

Two main groups of starch cells were distinguished. *Group A* (broken cells) were cells of any color with a greater or smaller fracture. Most of these were wholly stained red, although some were found where only the splitting surface of a crushed cell or just a portion associated with the fracture had turned red, while the rest of the cell remained blue. This group included also a few cells which, being hemispherical, resembled halved cells but were entirely blue. The number of these cells did not seem to be increased by grinding, and nothing definite can be said as to their origin. *Group B* (red-stained cells) were cells which were stained either entirely or partly red. A small part of these

were visibly broken and consequently belonged also to group *A*. In most of the red cells no visible fracture or other mark of external injury could be detected.

The results of the microscopic investigation are presented in Table V, which shows the total number of counted cells, as well as the percentages of the differently changed cells of the *A* and *B* groups, in terms of the total. For convenience only such cells as were at least 20 μ in diameter (long) were counted. It may be mentioned that as a rule all cells below that size were stained entirely blue and appeared perfectly intact. To make the comparison easier, the results are also illustrated graphically in Figure 2.

TABLE V

INJURED STARCH CELLS AS PERCENTAGES OF THE TOTAL NUMBER OF CELLS 20 MICRONS OR LARGER IN DIAMETER

A = broken cells
B = cells stainable with Congo red

Material		Maltose after 1 hr. at 30° C. (dry basis)	Starch cells		
No.	Ground to pass through		Cells ($\geqq 20\mu$) counted	A Broken cells	B Red cells
		%	*No.*	%	%
0	(Original material)	0.97	8372	1.85	4.43
1	60 g.g.	1.02	7857	2.05	5.96
2	6 xx	1.22	5333	2.47	8.37
3	10 xx	1.82	5226	3.02	17.9
4	14 xx	2.67	4696	3.02	23.2
5	25 xx	4.07	7018	3.31	29.2
E	25 xx (overground)	5.93	6339	3.77	42.1

Table V presents some very interesting data. First of all, the number of broken cells (group *A*) is augmented very little with the increased grinding, in any case too slightly to explain adequately the rapid increase in amylolytic activity. Especially astonishing it is to note that even deliberate "overgrinding," as described above, has not increased the number of broken cells more than 100% whereas the maltose figure has become six times the original (Table V, materials No. 0 and *E*).

The red-stained cells (*B* group), most of which are seemingly perfectly uninjured, play probably a more important role in the increase of amylolytic activity. This assumption is supported by the fact that the group *B* (Table V and Fig. 2) increases with great rapidity as the degree of fineness advances, even more rapidly than do the maltose

values. The justification for this supposition is confirmed also by the following experiment: Starch washed from material No. 3 was treated with a rather strong diastase preparation. The original material contained 3.0% cells of *A* group and 17.9% of *B* group. After the enzyme treatment the number of the cells of the *A* group had risen to

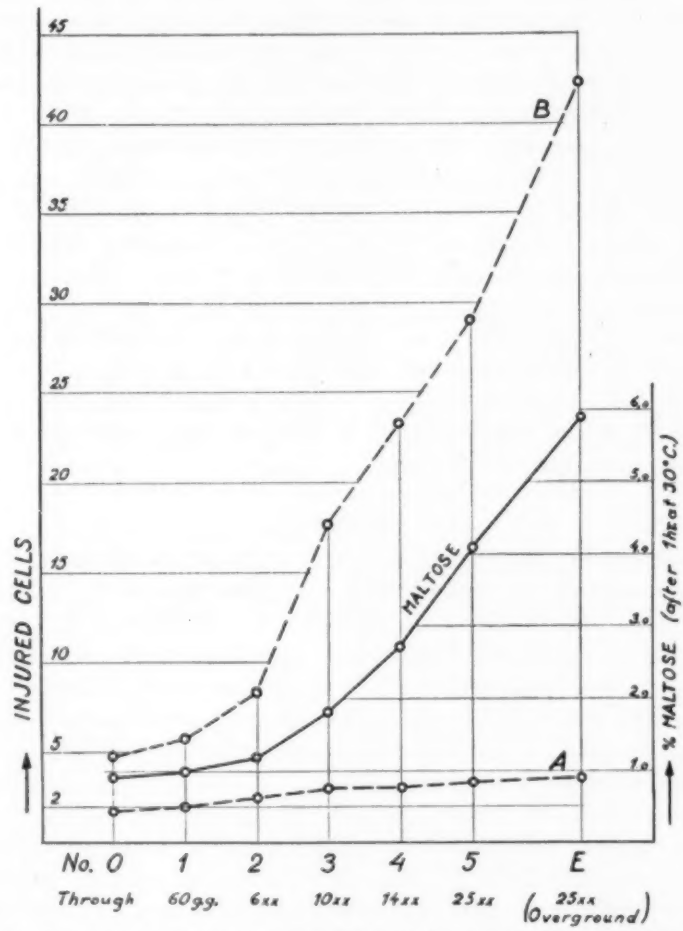

Fig. 2. Different types of injured starch cells in relation to maltose figures. (See Table V.) A—Percentages of broken cells of the total number of cells ≧ 20µ in diam. B—Percentages of cells stainable with Congo red, of the total number of cells ≧ 20µ.

13.5%, whereas the new counting gave for the *B* cells practically the same result (17.1%) as before the treatment. This experiment shows that the red-stainable cells, though seemingly entire, are very susceptible to the action of the amylolytic enzymes. It is to be remembered that this great change in the behavior of starch has been effected by a purely mechanical treatment.

Perhaps the only assumption permitting one to explain both the last-mentioned phenomenon and the above-described observations pertaining to the staining of cells is that the starch cells of wheat are surrounded by a separate thin layer or a film, which can be easily removed by mechanical means, so that the inside of the cell is exposed to the amylase effect. By supposing now that the film cover, in contrast with the substance under the film, is not stainable with Congo red, and that it is, like the rest of the cell, stained blue with iodine, it is very easy to understand why some of the fractured cells were, as described above, colored partly red and partly blue.

The assumption that the starch cells of wheat were coated by a thin filmlike layer of a substance which can be removed by mechanical treatment would be in good accordance with the conception Weichsel (1937) presents on potato starch. This investigator describes, for example, some experiments where the film is made visible by drying the starch over P_2O_5 for a very long time. In regard to other starch varieties, *e.g.* wheat starch, any direct evidence has not been presented, though it is usually supposed that these cells also are covered by a filmlike structure of amylopectin. In any case, it is certain that the outer part of the starch granule is in its physical structure different from the main body of the cell.

Summary

Some effects of particle size on different baking characteristics and on the properties of gluten and starch of wheat have been investigated. In all experiments the same semolina and middlings material was used, and all results and suggestions apply only to this material.

The material was reduced to various degrees of fineness, to pass through the following sieve numbers: 60 g.g., 6 xx, 10 xx, 14 xx, and 25 xx. In grinding, any elevation of temperature, likely to cause chemical changes, was prevented.

The medium-fine flour (through 10 xx) showed definitely better baking properties than the coarser and finer products. The coarsest (original) and the finest (through 25 xx) materials gave smaller loaves, and the qualities of crust and crumb were inferior; *e.g.*, the color of crumb was grayish.

The gluten test of Berliner and Koopmann gave reliable results, but was rather unsensitive for this special purpose. The farinographic method was not sensitive enough, and the standard diagrams for the coarse materials were not in good accordance with the results obtained by baking tests and other methods. The adaptability of this method to comparison of the so-called "granular flours" with the ordinary types of wheat flour is questionable.

The reduction of the size of particles increased water absorptions substantially; the periods of dough development (farinographic) were simultaneously lengthened.

With increasing degree of fineness great increases in the amylolytic activity (saccharogenesis) were noted. Two different methods, the maltose test of Blish and Sandstedt and the fermentograph method (Brabender), were employed. The results obtained and the characteristics of these methods are compared.

An assumption is advanced (supported by experimental evidence) that the starch cells of wheat are surrounded by a film or a thin layer of substance which is easily removable by means of mechanical treatment. The film is colored blue with iodine, but, unlike the main part of the cell, it is not stainable with Congo red.

Literature Cited

Alsberg, C. L., and Griffing, E. P.
1925 Effect of fine grinding upon flour. Cereal Chem. **2**: 325–344.
Haltmeier, O.
1928 Nummern und lichte Maschenweiten von Siebbespannungen. Z. ges. Mühlenw. **5**: 7–11.
Karacsonyi, L. P., and Bailey, C. H.
1930 Relation of the overgrinding of flour to dough fermentation. Cereal Chem. **7**: 571–587.
Kozmin, Natalie P.
Das Problem der Backfähigkeit, p. 31. (Moritz Schäfer, Leipzig.)
Kress, C. B.
1929 Granulation of flour and its relation to baking quality. Cereal Chem. **6**: 202–214.
Le Clerc, J. A., Wessling, H. L., Bailey, L. H., and Gordon, W. O.
1919 Composition and baking value of different particles of flour. Operative Miller **24**: 257–258 (reviewed by Shollenberger and Coleman, 1926).
Markley, Max C.
1934 Flour particle size by the sedimentation method. Cereal Chem. **11**: 654–660.
Pulkki, L. H.
1938 Über die Einwirkung des Ausmahlungsgrades und der Vermahlungsmethodik auf die Mehlbeschaffenheit. Mühlenlab. **8**: 49–52.
Shollenberger, J. H., and Coleman, D. A.
1926 Influence of granulation on chemical composition and baking quality of flour. U. S. Dept. Agr. Bul. 1463.
Shollenberger, J. H., Marshall, W. K., and Hayes, J. F.
1921 Influence of the size of flour particles on baking quality. National Miller, Dec., pp. 29–31 and 66 (reviewed by Shollenberger and Coleman, 1926).
Van der Lee, G.
1928 Die Bedeutung des Feinheitsgrades des Mehles für die Müllerei und Bäckerei. Z. ges. Getreidew. **15**: 78–85.
Weichsel, Gertrud
1937 Der diastatische Abbau nativer Kartoffelstärke. Planta **26**: 28–47.

THE SOLUBILITY OF SOME CONSTITUENTS OF SOYBEAN MEAL IN ALCOHOL-WATER SOLUTIONS

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This communication presents solubility data for the nitrogenous compounds, lecithins, and sugars of defatted soybean meal in various alcohol-water solutions and describes the effect of these solutions upon the subsequent water dispersion of the residual meal.

Mashino (1929) extracted defatted soybean meal with both methanol- and ethanol-water solutions of various concentrations. His object was to purify the meal in order to make it more palatable as human food. His results are in fairly close agreement with those presented here for the range of alcohol concentration greater than 60%. For more dilute alcohol solutions his results indicate much lower solubilities (50% or less of the nitrogenous material) than those reported in this paper.

Several publications mention the action of alcohol upon soybean proteins. Okano and Ninomiya (1929) state that an alcohol extraction decreases the amount of protein soluble in subsequent extractions by both water and sodium chloride solutions. No quantitative data showing the extent of this effect were reported. Izume and Yoshimaru (1931) also noted the denaturing effect of alcohol upon soybean meal. They reported that the protein in soybean cake, produced by extraction with hot alcohol, was soluble in water to the extent of 24% while the protein of the untreated bean was soluble to the extent of 88%. Alcohol and alcohol mixtures have been used for many years in the Orient to extract the oil from soybeans on a commercial scale, and it has been claimed by Bollmann (1923) that similar methods have commercial possibilities in the United States, especially in the recovery of byproducts such as lecithin.

Experimental

The soybeans used in the present investigation were of the Illini and Dunfield varieties. The Illini beans used for most of this work were grown during 1936 near St. Joseph, Illinois. The Dunfield beans

[1] A cooperative organization participated in by the Bureaus of Chemistry and Soils and Plant Industry of the U. S. Department of Agriculture, and the Agricultural Experiment Stations of Illinois, Indiana, Iowa, Kansas, Michigan, Minnesota, Missouri, Nebraska, North Dakota, Ohio, South Dakota, and Wisconsin.

were grown near Lafayette, Indiana, during 1937. The beans were flaked, extracted with petroleum ether, and ground in a ball mill to pass through a 100-mesh sieve. A 2.5-gram sample was extracted by shaking at room temperature (approximately 25° C.) for 30 minutes with 100 cc. of the alcohol-water solution. The suspension was then centrifuged and the supernatant liquid decanted into a digestion flask. The nitrogen was determined by the Kjeldahl-Gunning-Arnold method. A separate determination for nitrogen was made on each of the three successive extractions. All determinations were made in duplicate and checked by a later analysis, also in duplicate. Most duplicate determinations agreed very closely, and none were accepted that differed by more than 1%. The results reported are the averages of duplicate determinations. Alcohol concentrations are given in per-

TABLE I

PERCENTAGES OF TOTAL NITROGEN[1] EXTRACTED BY METHANOL-WATER SOLUTIONS AND BY WATER ALONE

Number of extraction	Nitrogen extracted by methanol-water solutions, percentage methanol being—										Nitrogen extracted by water
	100[2]	80	65	50	40	35	30	25	20	10	
	%	%	%	%	%	%	%	%	%	%	%
First	1.08	2.44	3.39	8.0	14.8	19.2	25.0	40.3	58.4	74.9	78.9
Second	0.35	0.48	0.64	1.7	2.8	4.2	10.0	19.5	21.7	14.1	11.6
Third	0.19	0.14	0.22	0.5	1.4	6.0	10.5	14.0	5.1	2.5	2.2
Total	1.62	3.06	4.25	10.2	19.0	29.4	45.5	73.8	85.2	91.5	92.7

[1] Percentage of nitrogen is based on 80.08 mg. per gram of meal = 100%.
[2] Commercial absolute methanol.

TABLE II

PERCENTAGES OF TOTAL NITROGEN[1] EXTRACTED BY ETHANOL-WATER SOLUTIONS AND BY WATER ALONE

Number of extraction	Nitrogen extracted by ethanol-water solutions, percentage ethanol being—										Nitrogen extracted by water
	100[2]	90	70	60	45	35	30	25	20	10	
	%	%	%	%	%	%	%	%	%	%	%
First	0.23	1.20	2.83	3.94	8.4	13.5	21.8	30.2	44.0	70.6	78.9
Second	0.17	0.43	0.49	0.92	1.7	3.3	7.4	14.8	26.2	16.7	11.6
Third	0.07	0.23	0.22	0.36	0.6	1.2	7.3	10.9	10.6	2.2	2.2
Total	0.47	1.86	3.54	5.22	10.7	18.0	36.5	55.9	80.8	89.5	92.7

[1] Percentage of nitrogen is based on 80.08 mg. per gram of meal = 100%.
[2] Commercial absolute ethanol.

centage by volume. A more complete description and study of the above procedure has been published by Nagel, Becker, and Milner (1938). The above extraction procedure subjects the meal to mild treatment. In comparing the results here reported with those obtained using commercial soybean meal or flour, cognizance should be taken of the different histories of the material, particularly when the flour is made from expeller meal. Tables I and II present the data on soluble nitrogen for methanol and ethanol extractions, respectively.

For comparison the total amounts extracted by each alcohol are shown graphically in Figure 1. It may be noted that at any given

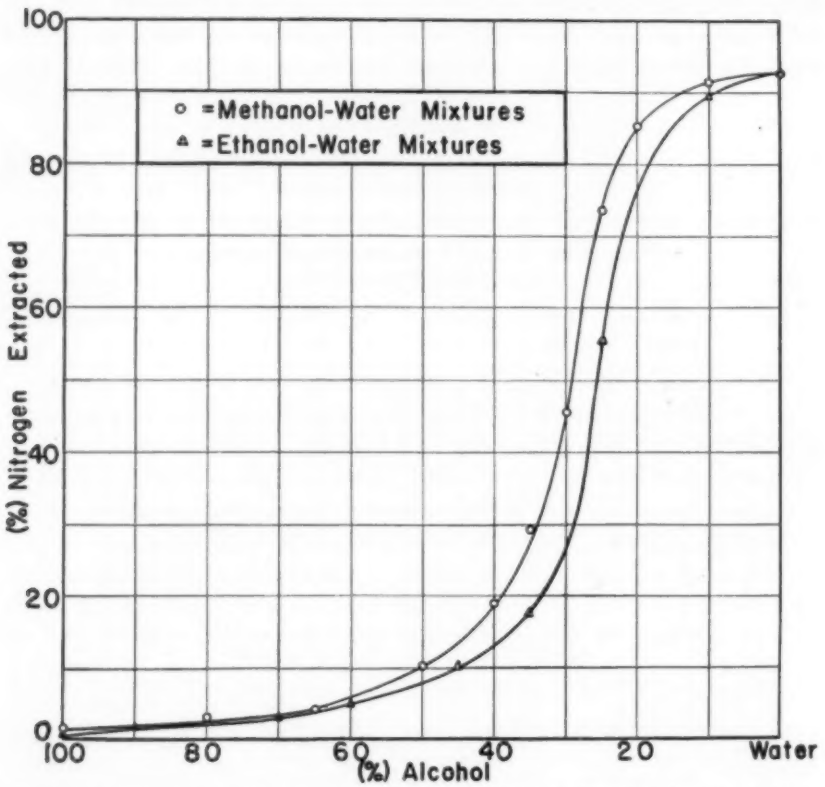

Fig. 1. Percentages of nitrogen extracted from defatted soybean meal by alcohol-water solutions.

alcohol concentration the amount of nitrogenous material extracted by methanol is slightly greater than that extracted by ethanol.

The maxima exhibited by both the second and third extracts in the dilute alcohol mixtures are noticeable in Tables I and II. These are illustrated graphically for the methanol extractions in Figure 2 in which each extract as well as the sum of the three extractions is plotted.

It can be noted from Figure 2 that the maxima shown by the second and third extracts are related to, and are probably a result of, the failure of the first extraction to remove the same proportion of nitrogen in this region that it does at other concentrations. This irregularity is indicated by the dotted curve in Figure 2, which shows that the

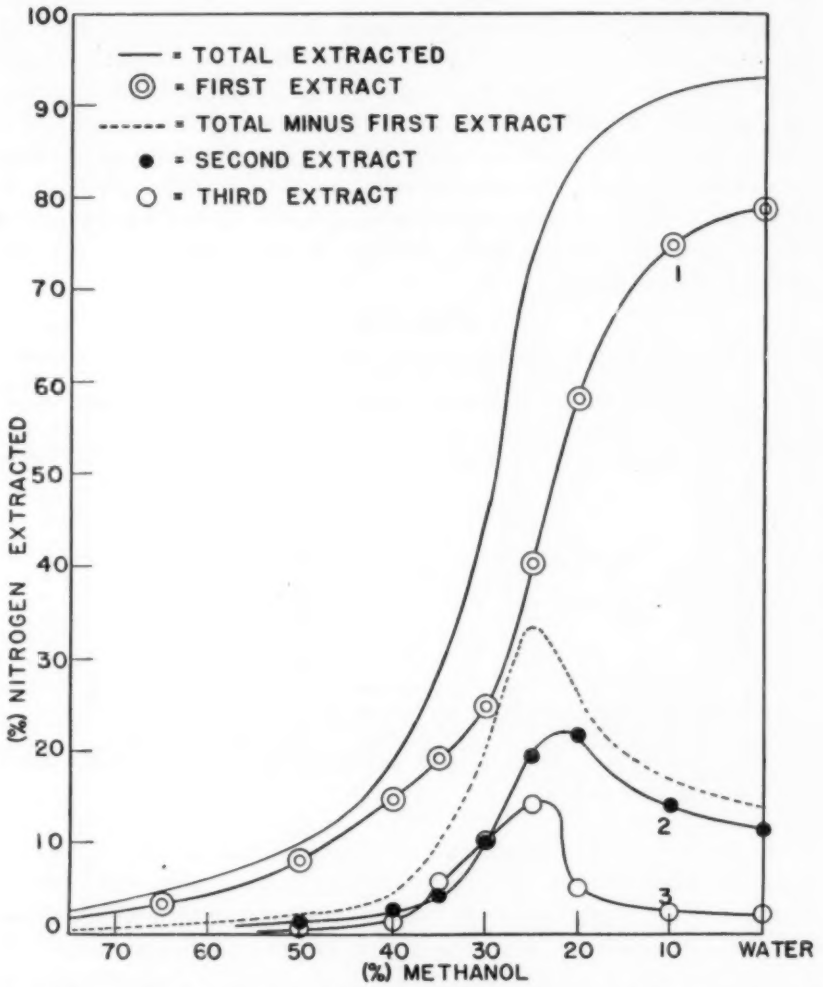

Fig. 2. Percentages of nitrogen extracted from defatted soybean meal by each of three successive methanol-water solutions.

first extraction leaves a varying percentage of available material for subsequent extractions. No explanation is offered here, but it is interesting to note that this phenomenon occurs in a region where there is a rapid change in the dispersability of the nitrogenous constituents.

Since the extraction of constituents from the meal by the alcohol-water mixtures might account for the changed properties of the meal, the alcohol-water extract was analyzed. Such an extract would be expected to contain phosphatides and carbohydrates. The phosphatides as a group are difficult to determine quantitatively. Since the phosphatides are very similar to each other in properties, a determination of any one of them, such as lecithin, may serve to indicate the presence of the others. Lecithin yields choline on hydrolysis and choline may be satisfactorily determined by Roman's method (1930). An aliquot of the alcohol extract, when hydrolyzed for 16 hours with boiling 5N sulfuric acid, yielded reproducible amounts of choline. Total sugars were determined according to the method of the A. O. A. C. (1935). Both of these analyses were made on the ethanol-water extract obtained in the same manner as described above for the nitrogen analysis.

TABLE III

PERCENTAGES OF CHOLINE AND SUGAR EXTRACTED BY ALCOHOL, AND NITROGEN EXTRACTED BY WATER FOLLOWING ALCOHOL EXTRACTION

Concentration of ethanol	Choline[1]	Sugar[2]	Nitrogen[3]
%	%	%	%
100[4]	23.4	5.3	88.2
95	35.3	16.9	87.8
80	68.3	80.6	74.0
70	71.0	87.9	62.8
60	71.0	90.9	63.5
50	50.8	91.9	68.2
40	46.8	92.5	72.3
30	——	91.3	——

[1] Percentage of choline is based on 2.52 mg. per gram of meal = 100%.
[2] Percentage of sugar is based on 97.32 mg. per gram of meal = 100%.
[3] Percentage of nitrogen is based on 80.08 mg. per gram of meal = 100%. Percentage of nitrogen extracted by three water extractions of the residue after one extraction with an alcohol-water solution of the concentration shown on the left.
[4] Commercial absolute ethanol.

In order to obtain quantitative information of the extent of alcoholic denaturation, three separate extractions, each with 100 c.c. of water, were made upon the meal remaining after one extraction with an alcohol-water mixture. These values and the results of the choline and sugar determinations of these extracts are presented in Table III. For convenience of comparison, the results from Table III and the nitrogen determinations for the first alcoholic extract shown in Table II are presented graphically in Figure 3. The dotted curve, 1*B*, in Figure 3 was obtained by calculating the percentage of available or remaining nitrogen removed by water after the alcoholic extraction. The dotted curve therefore gives a more accurate picture

of the extent of denaturation than curve 1*A*, because the results shown in 1*B* have been corrected for the amounts of nitrogen removed by the alcoholic extractions.

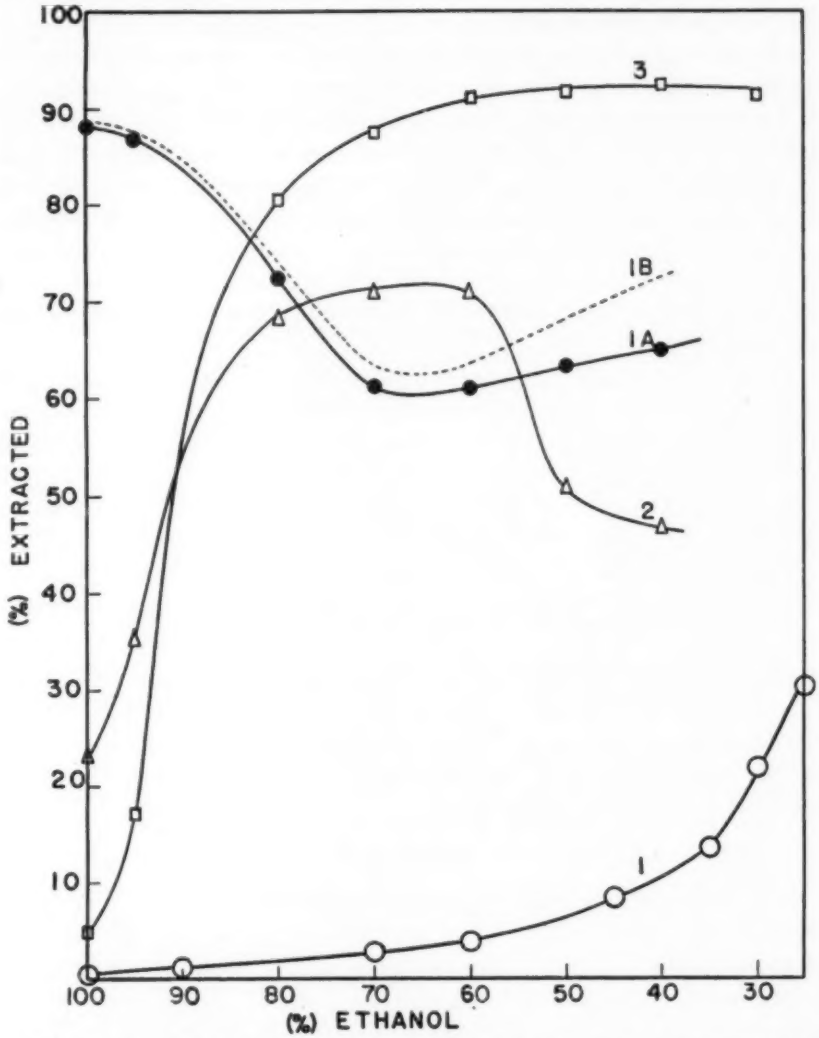

Fig. 3. Curves 1, 2, and 3 represent the percentages of nitrogen, choline, and sugar, respectively, removed by one extraction with ethanol-water solutions from defatted soybean meal. Curve 1*A* represents the percentages of nitrogen removed by three successive water extractions upon the residue from the corresponding alcohol extraction. Curve 1*B* is 1*A* corrected for the nitrogen removed by the alcohol extraction.

The effect of time of contact of the alcohol-water solutions on the meal was investigated. Seventy percent alcohol was used to make the initial denaturing extraction, followed by three water extractions each

for a 30-minute period. The results of these extractions are presented in Table IV. The soybeans used for these experiments were of the Dunfield variety. The amounts of nitrogen extracted by 70% alcohol and the subsequent water extractions agree very closely with those of the previously used sample.

In order to test the effect of adding lecithin to replace that removed by the alcohol extraction, the following experiment was carried out. A 2.5-gram sample of meal was extracted with 70% alcohol and the amount of lecithin removed was calculated from the choline found. Soybean lecithin, carefully extracted here, in amount equal to that removed by the alcohol, was suspended in 100 c.c. of water and the

TABLE IV

EFFECT OF TIME OF CONTACT BETWEEN 70 PERCENT ALCOHOL AND SOYBEAN MEAL UPON THE SUBSEQUENT WATER SOLUBILITY

Number of extraction	Nitrogen[1] removed by water from soybean meal after contact with 70% alcohol for—					
	0.25 hr.	0.5 hr.	2.0 hrs.	4.0 hrs.	24.0 hrs.	50.0 hrs.
	%	%	%	%	%	%
First	56.7	55.7	48.8	43.8	28.5	19.8
Second	9.2	9.9	13.1	15.0	22.5	19.7
Third	1.9	1.6	4.2	3.0	6.8	8.2
Total	67.8	67.2	66.1	61.8	57.8	47.7

[1] Percentage of nitrogen is based on 77.0 mg. per gram meal = 100%.

alcohol-extracted meal extracted with this lecithin-water mixture. It was found that lecithin added in this way had no effect in restoring the tendency of the nitrogenous constituents to go into solution; that is, the dispersability of the alcohol-denatured meal was the same whether lecithin was added or not.

Discussion

Choline is a degradation product of lecithin; hence for any given lecithin the choline constituent accounts for a certain definite proportion of the lecithin molecule. The exact factor expressing the relationship between the molecular weights of choline and lecithin varies slightly with the fatty-acid constituents in the lecithin molecule. Assuming that soybean lecithin has an average molecular weight of 784.7, the ratio between 784.7 and the molecular weight of choline, *i.e.* 121.2, is 6.47 to 1. Using this value and the 2.52 mg. of choline per gram of meal reported in Table III, the lecithin content of the meal is 16.30 mg. per gram. In general this value is in agreement with that reported in the literature; for example, Bailey, Capen, and

LeClerc (1935) found 1 to 1.5% lecithin in soybean flour, and Oberhard and Nechaev (1933) reported 1.5 to 1.6% in defatted soybean meal.

In Figure 3 a correlation may be noted between the amount of choline or lecithin removed (curve 2) and the extent of denaturation as indicated by the water dispersion of the alcohol-treated meal. The effect of various inorganic ions and variable pH on the dispersion of the meal has been considered by Smith and Circle (1938). It is recognized that there are other substances such as saponins, etc., that might partially account for the ease and completeness with which soybean proteins disperse into water, but it may also be stated that if lecithin were the dispersing agent such a relationship as has been noted between the above-mentioned curves would logically be expected.

In support of this theory it may be pointed out that the dispersing power of lecithin is well recognized; in fact the commercial utilization of it is based largely on this property (Wieshahn, 1937). Lecithin is present in soybean meal in sufficient amounts to be effective as a dispersing agent, and it is in intimate contact with—or perhaps a part of it is in chemical combination with—the protein. Blish (1936) has suggested that a lecitho-protein is the emulsifying agent in wheat bread dough. He also presented evidence that this complex is easily split by alcohol, and attributes the denaturation of wheat protein to this action. Sell, Olsen, and Kremers (1935) showed that a lecitho-protein is the emulsifying and stabilizing agent of egg yolk in making mayonnaise.

Horvath (1937) speculated upon the possibility of protein-phosphatide combinations in soybeans, mentioning both cephalin and lecithin as the specific phosphatides involved.

The fact that added lecithin does not restore the original water dispersability is probably an indication of the intimate relationship between the protein and lecithin originally present. This relationship could be either close physical proximity or actual chemical combination. In either case the alcohol apparently irreversibly removes the lecithin from a state which favors water dispersion of the protein. The fact that longer time causes greater denaturation as shown in Table IV further supports this view.

Another point that may be noted in Figure 3 is that the dehydrating action of alcohol is apparently not the factor causing denaturation. Absolute alcohol, a vigorous dehydrating agent, has much less effect upon the subsequent water solubility of soybean meal than 60% alcohol. Figure 3 also gives no indication that the amount of sugar extracted by the alcohol-water mixture is related to the observed denaturation.

Summary

The solubility of the nitrogenous constituents of soybean meal in both methanol- and ethanol-water solutions has been determined.

The denaturing action of ethanol-water solutions upon soybean protein was investigated.

The solubility of soybean lecithin in ethanol-water solutions has been determined, and the quantity extracted correlated with the observed denaturation.

The quantity of sugars extracted from soybean meal by means of ethanol-water solutions has been determined.

It is suggested that the lecithin present in soybean meal is largely responsible for the ease and completeness with which soybean protein disperses into water.

Literature Cited

The Association of Official Agricultural Chemists
1935 Methods of analysis of the association of official agricultural chemists. Fourth ed.: 341.

Bailey, L. H., Capen, R. G., and LeClerc, J. A.
1935 The composition and characteristics of soybeans, soybean flour, and soybean bread. Cereal Chem. **12**: 441–472.

Blish, M. J.
1936 The wheat flour proteins. Cereal Chem. **13** (supplement): 16–23.

Bollmann, Hermann
1923 Process of obtaining lecithin from vegetable raw materials. U. S. Patent No. 1,464,557.

Horvath, A. A.
1937 The chemistry of soybean protein extraction. Chemistry and Industry **56**: 735–738.

Izume, Seiichi, and Yoshimaru, Yoshinori
1931 Soybean oil cake as a food and its nutritive value. I. The oil extraction process and the digestion coefficient of the proteins. J. Agr. Chem. Soc. Japan **7**: 87–96.

Mashino, Minoru
1929 On the purification of soya bean protein. J. Soc. Chem. Ind. Japan **32** (supplementary binding): 312–313.

Nagel, R. H., Becker, H. C., and Milner, R. T.
1938 Some physical factors affecting the dispersion of soybean proteins in water. Cereal Chem. **15**: 463–471.

Oberhard, N. A., and Nechaev, D. A.
1933 Lecithin from soybeans. Schriften Zentral. Biochem. Forschungs inst. Nahr, Genussmittelind (Moscow) **2**: 400–409.

Okano, Koji, and Ninomiya, Manoru
1929 Denaturing of protein of soybean by alcholic extraction. I. Abstracts from reports of the central laboratory S. Manchuria Railway Co.: 7–9.

Roman, Wadin
1930 A chemical method for the quantitative estimation of choline and some physical-chemical data for choline and its salts. Biochem. Z. **219**: 218-231.

Smith, A. K., and Circle, S. J.
1938 Peptization of soybean proteins: the extraction of nitrogenous constituents from oil-free meal by acids and bases with and without added salts. Ind. Eng. Chem. (in press).

Sell, H. M., Olsen, A. G., Kremers, R. E.
1935 Lecitho-protein, the emulsifying ingredient in egg yolk. Ind. Eng. Chem. **27**: 1222–1223.

Wieshahn, G. A.
1937 Phosphatides and their uses (a review). Oil and Soap **14**: 119–122.

A PRACTICAL METHOD OF OBTAINING PERMANENT RECORDS OF BAKING STUDIES

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(Read at the Annual Meeting, May 1938)

The camera is serving a useful purpose as a means of making permanent records of cereal products. Its ability to register accurately the external characteristics of the baked product is unquestionable. However, it has definite limitations when used to register internal characteristics.

Many methods, in addition to photography, have been used to record the characteristics of bread and cake. The most popular of these are pointed out by Heald (1929). First, there is the method of marking around the outside of a slice of bread, showing the area of a cross section. Here at the Institute this has been extended to include a free-hand drawing of the important internal characteristics of the slice. A second method consists of marking the outline of the bread by putting on a thin coat of ink and then stamping the loaf or slice on paper.[1] In experiments carried out by the author, this has been much less successful than the first method. Both of these methods are greatly inferior to photography. For details of the photographic method one may consult the papers of Harrel (1930), Cooley and Davies (1936), and Heald (1929).

Now, if one studies good photographs of the crumb of bread or cake, just how much do they show? Photographs definitely show some characteristics of the grain. They show holes, openness, and of course uniformity; however, they do not show the thickness of the cell walls or the cell structure. As to texture, photographs do not show denseness around the crust, lumpiness, cores, or ridges. However, comparative photographs (having a standard for comparison) give some idea of roughness and harshness.

Separate photographs without a standard for comparison will tell absolutely nothing concerning roughness, harshness, and crumb color. This is due to the many variables with which one has to deal. Harrel (1930) has pointed out how different lighting and different photographic emulsions affect the color and degree of harshness of the texture. By changing these variables the same loaf can be made to appear entirely different.

[1] See Child and Purdy (1926) and Mohs (1924).

The internal characteristics, which it might be possible for photography to show and which the baker is extremely interested in, are, in order of importance, grain, color, and texture. Just what photography is able to show concerning these characteristics has already been mentioned. In order to add to the already existing photographic method the following methods have been devised.

Preliminary Experiments

The method used in the preliminary experiments shows the porosity of the crumb—that is, holes, openness, lumpiness, cores, ridges, and denseness around the crust. The commercial baker is extremely interested in these points; and in most cases camera photography completely fails to show them.

By holding a slice of bread up to bright, even daylight, the density of the crumb shows up very nicely. This is the principle used here. Light is transmitted through the slice and then caught on photographic paper. A negative is not used, and the darker parts of the picture obtained represent the parts of the slice that permit the most light to pass and *vice versa*. Figure 1 shows the arrangement used. The light is made even and diffused by using a frosted lamp (150 to 200 watts serves very well) and a frosted glass plate. Although it is not necessary, this method of lighting could perhaps be improved by multiple lamps instead of one large one and by a holopane-plate placed in the reflector.

In Figure 2, A and B, are shown pictures obtained by this method. The photographic paper used was Defender, glossy single-weight contact paper. In A, No. 5 contrast paper was used, while that in B was No. 4 medium hard. It will be noted that the more contrasting the paper, the more magnified are the defects. The area around the crust is dense; hence very little light passed through. In the center there is a large dense spot or core. The area of the slice which has the best characteristic of density is pointed out in the figure. Figures 3A and 3B were made exactly in the same way as Figure 2 but are of a different type of bread. The dense streak or ridge in the center is plainly visible. Other characteristics can be noted. The disadvantages of this method are that it records crumb characteristics and texture only in so far as they are reflected by the porosity of the slice. The slice of bread must be of uniform thickness, and slices to be compared must be exactly the same thickness. However, much information can be derived from these pictures, if one has had some experience in interpreting them. Markings at the bottom of each picture represent inches; thus the degree to which they are enlarged or reduced can be determined.

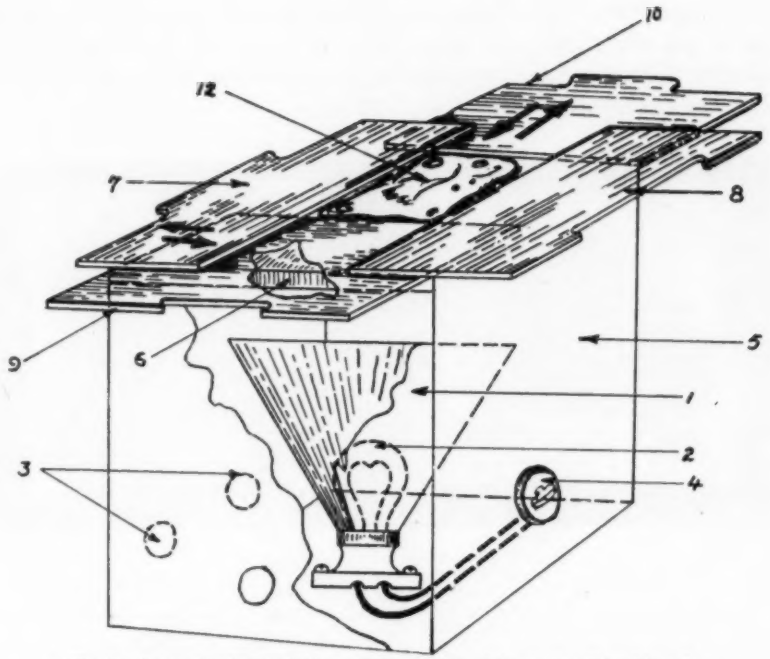


Fig. 1. Apparatus for producing contact shadow pictures of a slice of bread.

The reflector (1) is for the frosted light bulb (2), which, in turn, is operated by the switch (4). Air holes (3), for ventilation, are in the side of the metal box (5). The ground-glass plate (6) fits into grooves on the box. The metal slides (7, 8, 9, 10) (direction of motion shown by arrows) fit over the ground-glass plate (6) and are adjusted so as to cut out excessive light around the slice of bread (12), which rests on the ground glass plate (6).

Over the bread (12) is placed a thin, clear glass plate. Over the thin glass plate is placed the photographic paper, and then, in turn, another glass plate. The glass plates and photographic paper are not shown.

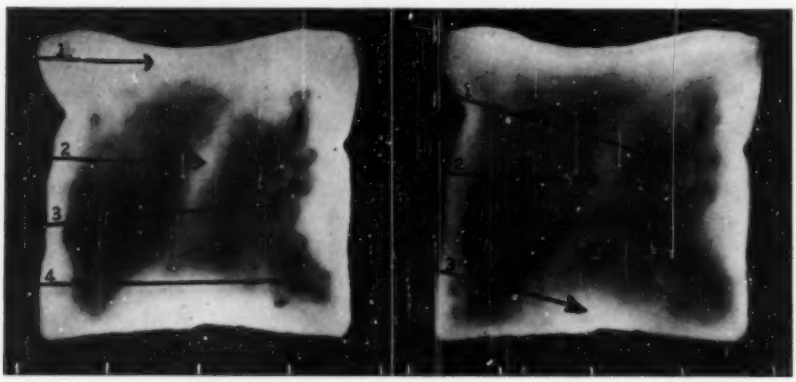
A B

Fig. 2. Contact pictures of white bread made by transmitting light through the slice. Figure 2A is made on contrast paper; Figure 2B on medium hard paper.

A: (1) Area showing best grain and texture of the slice. (2) Hole. (3) Porous area. (4) Dense area. (5) "Core" or dense spot.

B: (1) Area showing best grain and texture of the slice. (2) Dense area. (3) "Core" or dense spot. (4) Porous and open area.

The method of producing a shadow picture directly through the slice is not as satisfactory as another method, which will now be described. The new technique can be employed just as easily and brings out more of the characteristics of the slice.



A B

Fig. 3. Contact pictures of white bread made by transmitting light through the slice. Figure 3A is made on contrast paper; Figure 3B on medium hard paper.

A: (1) Dense area. (2) "Ridge" or dense section. (3) Area showing fair grain and texture but slightly porous and open. (4) Slighty porous and open area.
B: (1) Porous and open area. (2) "Ridged" or dense section. (3) Dense area.

The Method

Instead of catching the light which passes through the slice directly, on photographic paper, it is focused by means of a lens and the image is caught on photographic projection paper. At first the lens of a No. 2A Brownie box camera was used. The arrangement of the apparatus is shown in Figure 4.

Pictures made by this method are shown in Figure 5. Figure 5A was made with Defender, Velour Black, glossy, single-weight contrast projection paper, No. 44; 5B was the same except that No. 33 medium-hard paper was used. The pictures are of the same slice as those of Figure 2. It will be noticed that the medium-hard paper is the "softer" of the two and perhaps catches more of the edges; however, the contrast paper shows more detail (shows cell structure better), and is undoubtedly preferable. The contrast paper again magnified the defects more.

These pictures show the crumb characteristics and the texture without losing any of the factors of porosity shown by the foregoing method.

The denseness around the crust is still very evident. Although cell structure does not show up around the crust, this does not mean that the bread is solid in this region. The cells are there but the struc-

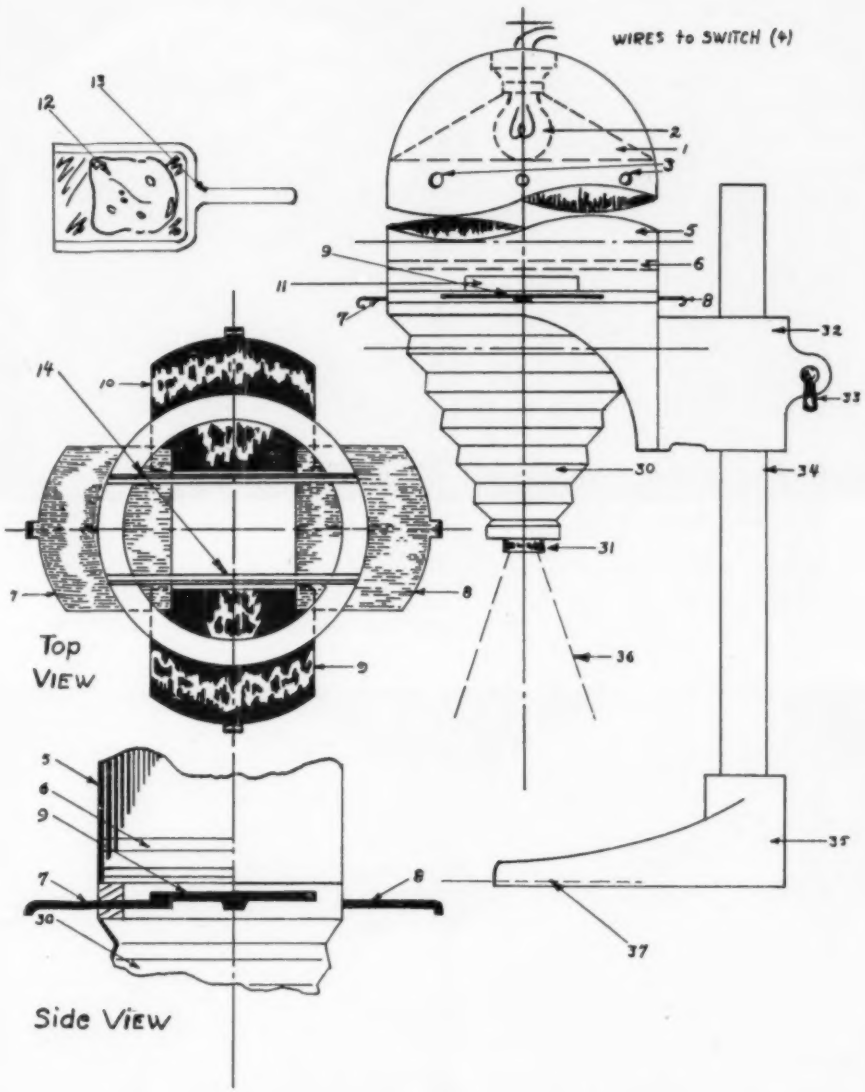
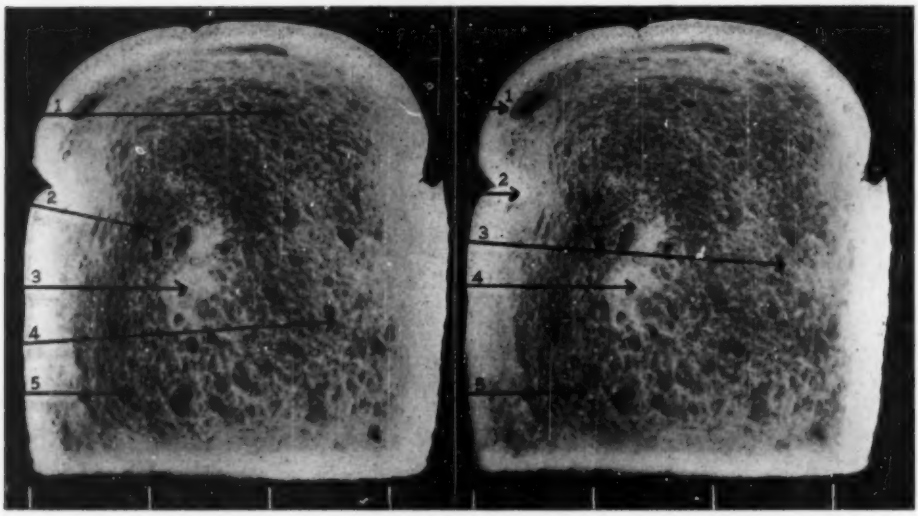


Fig. 4. Apparatus or producing focused projection pictures of a slice of bread.

The reflector (1) is for the frosted light bulb (2), which, in turn, is operated by the switch (4). Air holes (3), for ventilation, are in the side of the metal case (5). The ground-glass plate (6) fits in the case (5) (its position is adjusted up or down until the light is even and diffused). The metal slides (7, 8, 9, 10) (direction of motion similar to Figure 1) are adjusted so as to cut out excessive light around the slice of bread (12), which rests on the glass tray (13). The bread (12) and tray (13) fit into opening (11) on runners (14).

The bellows (30) are in permanent adjustment and hold the box camera lens (31). The apparatus is held in place and focusing is made possible by (32, 33, 34, and 35).

The beam of light (36), formed by the lens (31), forms an image on the projection paper, which, in turn, is held by a paper holder (37).

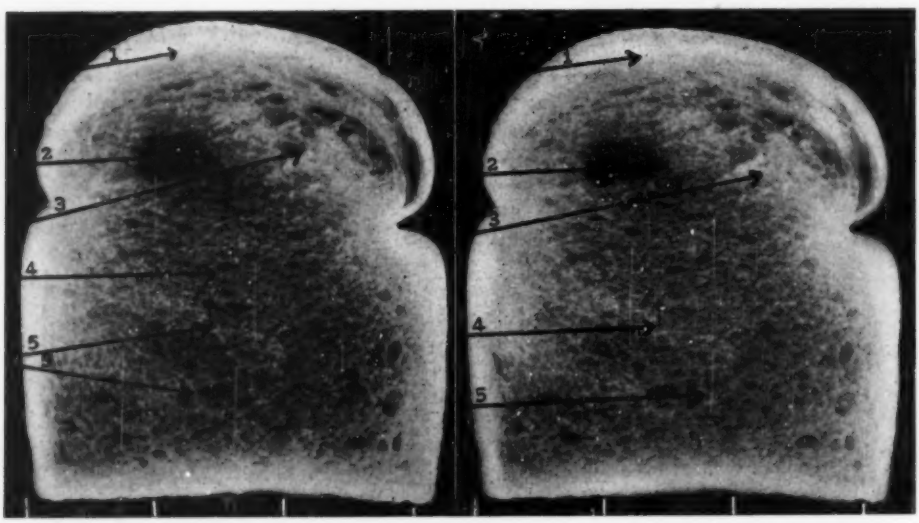


A B

Fig. 5. Pictures of white bread made with a photographic projection apparatus. A Brownie camera lens was used. Figure 5A is made on contrast paper, Figure 5B on medium hard paper.

A: (1) Area showing fair grain and texture. (2) Slightly open grain. (3) "Core" or dense spot. (4) Slightly dense and rough area. (5) Porous and open area.

B: (1) Hole. (2) Dense area. (3) Slightly dense and rough area. (4) "Core" or dense spot. (5) Porous and open area.



A B

Fig. 6. Pictures of white bread made with a photographic projection apparatus. A Brownie camera lens was used. Figure 6A was made on contrast paper, Figure 6B on medium hard paper.

A: (1) Dense area. (2) Hole. (3) Area showing harsh texture. (4) Area showing fair grain and texture. (5) Slightly "ridged" or slightly dense.

B: (1) Dense area. (2) Hole. (3) Area showing harsh texture. (4) Slightly "ridged" or slightly dense. (5) Area showing slightly open grain.

ture is so dense that light does not penetrate very well. This denseness around the crust is one thing the baker tries to eliminate, and these pictures give him a definite record of this factor.

The large core in the center is evident. The most porous and open structure will be observed to be to the left of this core.

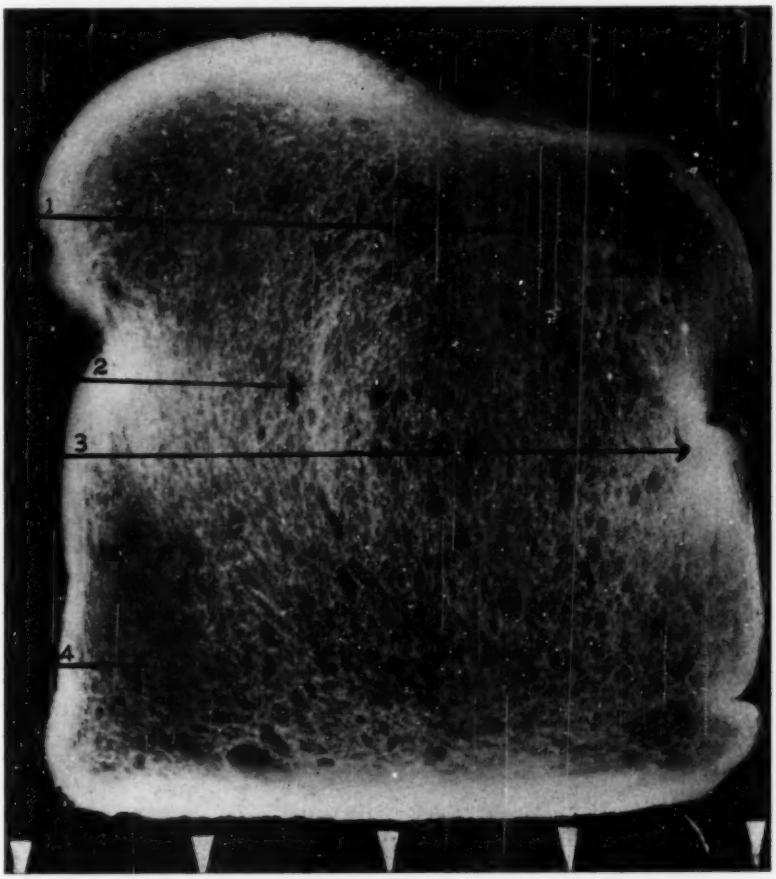


Fig. 7. Picture of white bread made with a projection printer. A projection anastigmat, f. 4.5 lens was used. It is made on contrast paper and is an example of very good grain and texture. (1) Slightly open grain area. (2) "Ridge." (3) Dense area. (4) Slightly open grain area.

The cell-wall structure is plainly visible and one should note the "depth of focus" in these pictures; they are not just surface exposures, as are the usual photographs.

Figures 6A and 6B are pictures of a slice from a different loaf, made in exactly the same way as Figures 5A and 5B. It will be noted that this is a somewhat better slice then the one before. The hole and open

section are evident. The rough, light surface near the top of the slice is due to a harsh texture. Ridges and other characteristics are pointed out in the figure.

It was believed that a lens especially adapted for this work would give an improved picture. With this in mind the pictures in Figures 7, 8, 9, and 10 were made with an Eastman projection printer. The projection printer was operated in the usual manner with the slice of

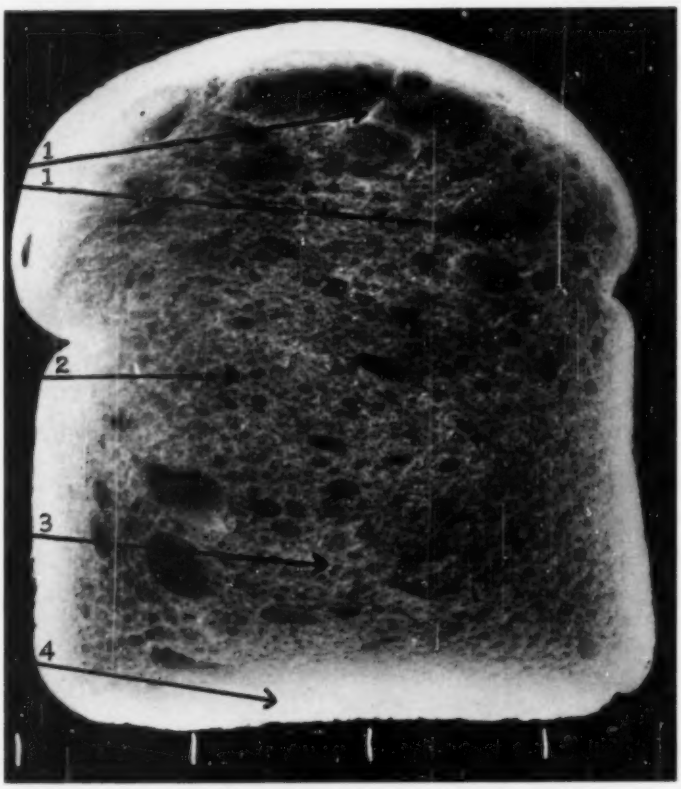


Fig. 8. Picture of white bread made with a projection printer. A projection anastigmat, f. 4.5 lens was used. It is made on contrast paper.
(1) Holes and open grain. (2) Good grain. (3) "Ridge." (4) Dense area.

bread replacing the negative. The lens was an Eastman projection anastigmat, f. 4.5 (price $50). The main advantages of this lens over the cheaper one are that it gives a sharper image, permits a shorter exposure, and the pictures are generally better.

This better lens can be used in the apparatus shown in Figure 4 in place of the Brownie camera lense. The whole set-up can be permanently adjusted, which would save time in making the pictures.

The expense of constructing this apparatus is less than the cost of a projection printer. In addition, a projection printer like the one mentioned above must be changed slightly because the opening for the negative is not large enough to accommodate a slice of bread.

Although in this method the slices must be of uniform thickness, it is not necessary to have different slices the same thickness in order to

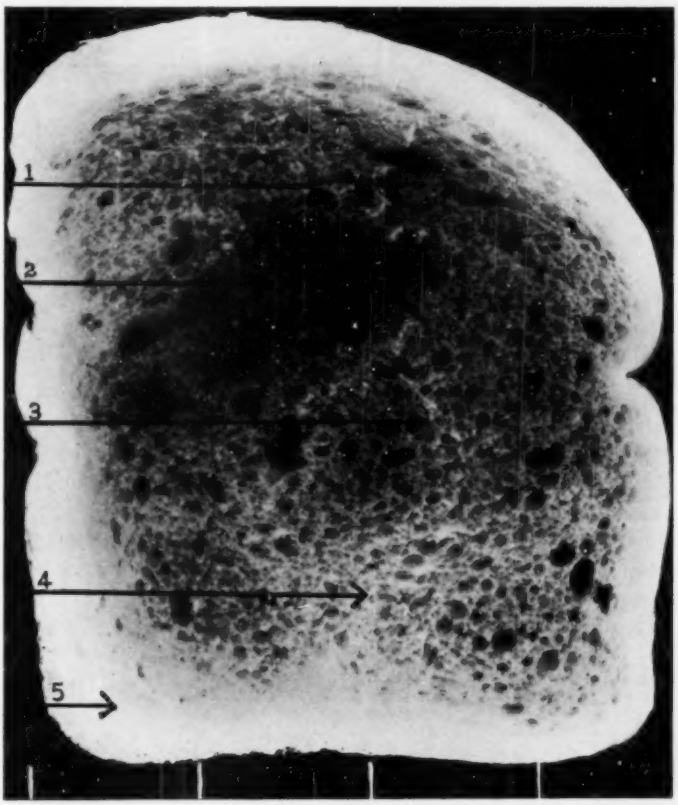


Fig. 9. Picture of white bread made with a projection printer. A projection anastigmat, f. 4.5 lens was used. It is made on contrast paper and is an example of very unsatisfactory grain and texture.

(1) Light spots due to dull cutting blades. (2) Area showing large holes and open grain. (3) Light spots due to dull cutting blades. (4) Slightly dense area. (5) Dense area.

obtain comparative pictures. A mechanical slicing machine is recommended; either the ordinary commercial bread slicer or the type used for slicing cold meats is suitable.

From the foregoing discussion the pictures are self-explanatory; however, a few interesting points will be mentioned.

Figure 7 is an example of very good bread. It has good crumb

characteristics and good texture, except for the ridge or streak which is noticeable. The thin cell walls are plainly visible. The bread shown in Figure 9 is in direct contrast to this. The grain is coarse and the cell walls thick. It is an example of unsatisfactory bread crumb. The quality of the breads of Figures 8 and 10 fall between those of Figures 7 and 9. The large holes show up plainly, and the depth of focus is evident by the cell structure visible in the holes. Dull cutting blades are evident in Figures 9 and 10.

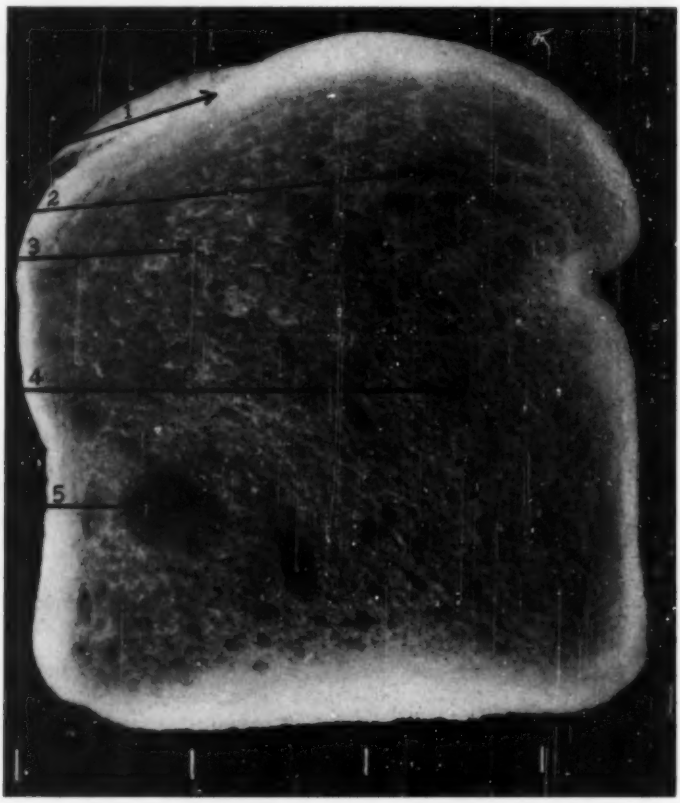


Fig. 10. Picture of white bread made with a projection printer. A projection anastigmat, f. 4.5 lens was used. It is made on contrast paper.
(1) Dense area. (2) Area showing open grain. (3) Light spots due to dull cutting blades. (4) Area showing good grain. (5) Hole.

Cost of Method

The cost of making each 5 x 6-inch picture is approximately four cents if 20 or more pictures are made, or about 8 cents separately. (These prices are for materials only.) The Eastman formula D-72 was used throughout this work as the developer and is entirely suitable.

The slices used to make the above pictures varied from $\frac{3}{8}$ to $\frac{9}{16}$ inch in thickness.

Other Uses of Method

Up to this point we have discussed only white bread. It is obvious that one would have trouble applying this method to dark breads, since they would permit only a little light to pass through the slice. Experiments with whole-wheat bread have not been very successful, even when the bread was sliced $\frac{3}{16}$ inch in thickness.

Pictures of pound cake are fairly satisfactory. However, the cake must be sliced $\frac{3}{16}$ inch in thickness or thinner. An example is shown in Figure 11.

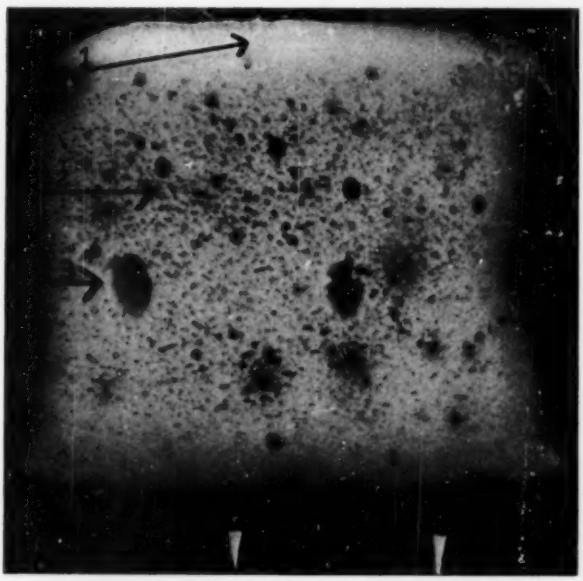


Fig. 11. Picture of yellow pound cake made with a projection printer. A projection anastigmat, f. 4.5 lens was used. It is made on contrast paper.
(1) Dense area. (2) Area showing open grain and texture. (3) Hole.

The procedure described above should prove valuable as a method of permanently recording the results of baking studies. It may have uses in many other fields.

Advantages of the Method

It magnifies the defects of the bread, thus making them more evident.

It more truly shows what the eye sees than does camera photography. It brings out detail below the actual surface.

A negative is not required, thus eliminating a step which causes variations in the results.

It shows more concerning the characteristics of grain and texture than does camera photography. It is believed that it shows all of the important characteristics of grain (uniformity, openness, thickness of cell structure and cell walls) and texture (roughness, harshness, lumpiness, cores, and ridges).

It can be used for both experimental and commercial loaves of bread.

It is cheaper, simpler, and requires less time than camera photography.

Fig. 12. "Photo-Record" camera constructed by The National Mfg. Co., Lincoln, Nebraska.

Summary

Preliminary experiments are described in which shadow pictures of slices of bread are obtained. Examples are shown.

A simple, direct method for recording the internal characteristics of a slice of bread is described. It utilizes the principle of photographic projection.

It is believed that the projection method records all of the important characteristics of the crumb and texture. Examples of various slices of bread (one for cakes) are given.

Supplement

Since this paper was prepared an apparatus similar to that described above has been constructed by the National Mfg. Co., Lincoln, Nebraska. It is obtainable from them. Two views of it are shown in Figure 12. The light arrangement is in the bottom section. The center section can be opened in order to put the slice of bread in place. The photographic projection paper is contained in the top section. A roll of paper is used to facilitate the operation and at the same time reduce the cost of the paper for each picture slightly under two cents. The "safety" glass window in the top front section, the opening on the left side, and the internal arrangement are such that the paper can be exposed without the aid of a dark room.

Acknowledgment

The author is indebted to William Walmsley and Andrew Habenicht for the drawing of Figures 1 and 4, to Doose's Studio for the use of their projection printer, and to several people in the baking and allied industries for reviewing the manuscript.

Literature Cited

Child, A. M., and Purdy, D. I.
1926 Method for a graphic record of texture, volume, and contour of cakes. Cereal Chem. **3**: 57–60.

Cooley, L., and Davies, J. R.
1936 The technique of photography for permanent records of baking studies. Cereal Chem. **13**: 613–620.

Harrel, C. G.
1930 Some basic principles of photography as applied to cereal work. Cereal Chem. **7**: 313–321.

Heald, W. L.
1929 A practical method of photographing bread. Cereal Chem. **6**: 69–71.

Mohs, K.
1924 The size of the pores in baked bread. Cereal Chem. **1**: 149–151.

MALTOSE FERMENTATION ACTIVATORS AS AFFECTING BAKING [1]

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(Read at the Annual Meeting, May 1938)

The importance of due recognition and control of the *gas production* factor in both the performance and interpretation of laboratory baking tests has received much emphasis in recent cereal technological investigations. Landis and Frey (1936) consider the regulation of the sugar level to be as important as the adjustment of absorption, or of any other item in the baking formula. The need for different adjustments for different flours is known to arise largely from variations in flour diastatic activity.

That there is a high but not absolute correlation between autolytic "maltose values" and actual gassing-power values has been shown by Blish, Sandstedt, and Astleford (1932), Davis and Worley (1934), Eva, Geddes, and Frisell (1937) and others. Karacsonyi and Bailey (1930) reported that although overgrinding gave increased autolytic maltose values it did not proportionally increase gas production. Although either method will serve for most practical purposes, there is every reason to regard the gassing-power test as more precisely informative than the autolytic maltose test.

As was shown by Blish, Sandstedt, and Astleford (1932) the variation in sucrose content of flours is in itself sufficient significantly to disturb the correlation between gassing power and autolytic maltose values. Sucrose does not register in the maltose test but does in gassing-power tests. By taking into account the diastatic activity and the original sucrose content of the flour it appears possible to adjust all flours to approximately equal dough sugar levels for baking purposes. Will such adjustments insure equal *rates* of gas production throughout fermentation with all flours? Our experience tends to justify a negative answer to this question, and to lend added support to the recommendation of an actual gassing-power method involving yeast fermentation as the only type of procedure suitable as a basis for controlling sugar levels by the addition of sugar or diastatic supplements.

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Eva, Geddes, and Frisell (1937) called special attention to the importance of gas production during the *later stages of fermentation*, and this is also clearly shown in the critical study reported by Landis and Frey (1936). Substantiating this viewpoint, our own experiences, especially with dough behavior during proofing, indicate that initial potential sugar level is by no means the only factor determining gas-production rate in the later stages of dough fermentation.

Flours sometimes show much greater differences in rate of proof than can be satisfactorily accounted for on the basis of either sugar levels or gas retention. It appears to be more a matter of *rate of fermentation* than of quantity of sugar available, assuming of course that there is no actual sugar *deficiency*. Our attention was first directed to this situation during the performance of cooperative baking tests on some special samples of Canadian flours [2] from wheats of the 1936 crop. These flours proofed at extremely high rates of speed as compared with other flours of equal and greater diastatic activities and sugar levels. It soon became evident that the conspicuous differences were due to variations not in gas *retaining* properties, but in *rate* of gas production.

The differences in behavior could be most plausibly explained as being manifestations of varying amounts of the specific maltose fermentation "activator" reported by Sandstedt and Blish (1934), by Genevois and Pavloff (1935), and by Blish and Sandstedt (1937). These reports show that flours do vary considerably in their ability to stimulate the fermentation of maltose by bakers' yeast, which by itself is frequently deficient in maltose fermenting potentiality. The experiments herein reported substantially confirm the foregoing explanation, and show beyond reasonable doubt that rate of gas production in the later stages of dough fermentation—as reflected in the time required to proof to a standard height—is very significantly influenced by the relative quantity of the maltose fermentation catalyst present in the flour.

Experimental

The plan of experimentation was to observe, in a series of flours, the degree of correlation between activator content and time required for proofing to standard height under comparable conditions affording adequate sugar levels in all doughs. The method of estimating relative amounts of "activator" in the individual flours was, with certain modifications, the procedure employed for the measurement of gassing power, using the "pressure meters" described by Sandstedt and Blish (1934). Two different modifications of procedure were tried, one

[2] Samples submitted by Dr. W. F. Geddes, of the Dominion Grain Research Laboratory.

designated as the "6-hour method" and the other the "3-hour method."

Six-hour method.—Ten grams of flour were made into a thin paste with 10 c.c. of water containing 0.3 gm. of yeast in suspension, and fermented for five hours in the pressure meter at 30°. This fermented out the sucrose originally present, as well as a large portion of the maltose produced by autolysis. At five hours the pressure-meter cups were opened, and 300 mgs. of maltose stirred into the paste with a spatula. Five minutes were given for the pressure-meter to again reach bath temperature and then fermentation was allowed to proceed for an additional hour, the pressure produced during the final (6th) hour being recorded as a measure of maltose fermentation rate. Differences in rate were considered to indicate corresponding variations in quantity of activator contained in the flours, and these values were correlated with time intervals, respectively, for doughs to proof to a height of 10 cm.

Three-hour method.—This method, a simpler procedure, differed from the preceding one in that 400 mgs. of maltose were added to the flour at the *beginning* of the gassing-power test. The pressures developed during the third hour of fermentation—obtained merely by subtracting the second- from the third-hour readings—were recorded. These values were correlated with the six-hour-method values on the one hand, and with proofing time on the other, as shown in Table I, the values for the three-hour method being arbitrarily placed in the order of descending magnitude.

Baking.—In baking the flours, the quantities of sugar used were adjusted in accordance with individual maltose values, the amounts being calculated to provide at least 5% of sugar available in the first hour. However, this control of sugar level must be considered to have been *approximate* only.

It appears from the "activator" values shown in Table I that flours vary considerably in their ability to stimulate maltose fermentation, and that this ability is not reflected by the gassing power of the flour itself. The variations are, however, reliably measured by either the three-hour or the six-hour method, both of which involve the addition of maltose. As the activator content decreases the proof time correspondingly increases. A few values are somewhat out of line—as for instance flour 24 and flour 31—but the general tendency is very pronounced and unmistakable. The few inconsistencies observed may have been due to either faulty control of sugar level or the factor of gas *retention*, or both. The highly positive nature of the correlation is graphically shown in Figure 1, in which activator values are plotted against proofing time values.

These findings and data should not be interpreted to mean that rate of gas production during the proofing period is governed *exclusively* by the quantity of activator present, for it is quite possible to influence the time required to proof to a definite height by adjustments of sugar level alone, especially in the lower sugar levels. Nevertheless, under

TABLE I

ORDER OF VALUES FOR MALTOSE FERMENTATION ACTIVATOR AS RELATED TO PROOFING TIME

Flour	Flour gassing power[1]	Activator units		Proof time[2]
		3-hr. method	6-hr. method	
		Mm. of mercury		*Minutes*
1	365	228	203	34
2	456	225	200	34
3	349	215	203	36
4	300	210	194	39
5	323	209	188	39
6	355	204	186	38
7	300	202	190	40
8	279	200	189	39
9	300	198	177	43
10	262	190	176	41
11	335	185	171	46
12	278	185	157	43
13	158	179	155	48
14	401	179	158	45
15	185	177	146	47
16	450	167	131	54
17	545	166	—	45
18	520	161	137	63
19	254	155	140	60
20	338	152	—	55
21	411	152	—	62
22	270	152	122	65
23	133	150	129	60
24	—	150	132	84
25	386	140	—	64
26	416	140	—	69
27	445	140	—	72
28	96	138	131	77
29	109	136	120	69
30	413	135	—	65
31	89	133	126	63
32	383	129	—	69
33	179	116	94	95

[1] Pressure in 4 hours, using 10 gms. of flour and 0.3 gm. of yeast.
[2] Proofed to height of 10 cm.

controlled conditions of adequate and *comparable* sugar levels, the flours containing the larger amounts of activator will stimulate maltose fermentation at correspondingly higher rates. Obviously, variations in quantity of activator are of no important consequence under conditions of pronounced sugar deficiency.

With the quantity of yeast and with all other conditions constant and controlled, it is found that for any individual flour there is a *maximum* rate of maltose fermentation that cannot be exceeded regardless of the amount of maltose present. It is this maximum rate that is determined and limited by the quantity of activator in the flour. This is typically illustrated by data given in Table II, involving gassing-power tests with two flours with additions of varying quantities of maltose. Ten grams of flour and 0.3 gram of yeast were used in these

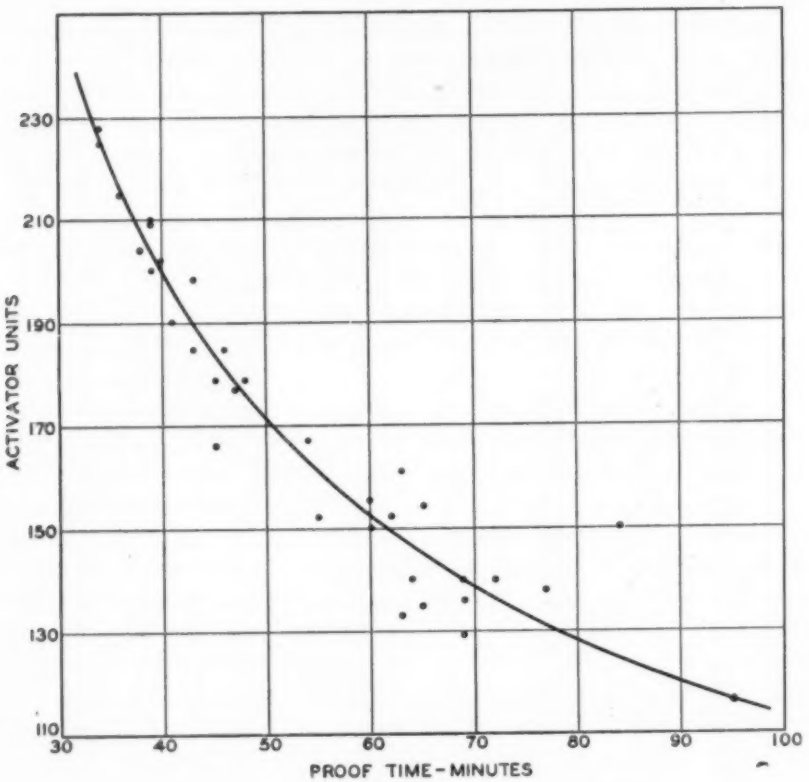

Fig. 1. Activator units plotted against proofing-time values.

determinations, with added amounts of maltose as indicated in Table II.

It is shown in Table II that although flour A had nearly twice the gassing power of B, its maximum rate of maltose fermentation was considerably less than that of B. Flour B, therefore, had a much higher activator content than A. Varying the amount of added maltose from 250 to 1000 mg. did not alter the fermentation rate for

flour A. For flour B, with a higher activator content, 250 mg. of added maltose was not enough to provide the maximum fermentation rate—due to the higher activator content combined with the low diastatic activity of the flour—but the rate was not changed by subsequent maltose additions from 500 to 1000 mg. The data show that, for the conditions employed, the maximum maltose fermentation rate for flour A is to that of flour B as 137 is to 184.

From the foregoing experiments and considerations it is to be concluded that the complete elimination of the *rate* of gas production as a variable in experimental baking is a difficult matter. Because of possible variations in activator content it appears that adjustment to approximately equal sugar levels will not necessarily insure equal *rates* of gas production. In the straight dough method of baking

TABLE II

MAXIMUM RATES OF MALTOSE FERMENTATION AFFORDED BY TWO FLOURS

Flour	Gassing power for flour alone[1]	Gassing rate with added maltose[2]			
		250 mgs.	500 mgs.	750 mgs.	1000 mgs.
	Mm. of mercury	*Mm. of mercury*			
A	450	137	137	138	136
B	235	148	185	183	184

[1] Four-hour reading.
[2] Pressure developed from 2nd to 3rd hour.

these rate differences are quite likely to be very pronounced during the proofing period. This very strongly suggests that unless and until it becomes possible to control the quantity of activator, itself, the most suitable *practical* method of making due allowance for this variable is to provide for an *adequate* sugar level and then proof all doughs to constant *height* rather than to constant *time*. By thus minimizing the importance of variations in amount of maltose fermentation activator one may reduce the probability of occasional unjust discriminations against flours having excellent baking utility.

Variations in the quantity of the maltose fermentation activator are, beyond all question, more likely to register significantly in straight dough than in sponge dough baking. In the latter case there is usually an ample supply of *sucrose* (added in dough stage) to provide all the gas needed during the proof period. Bakers' yeast does not require the presence of specific biological activators to ferment sucrose in the same sense or to anywhere near the same degree that these biocatalysts are needed for maltose fermentation.

The maltose fermentation activator is as yet unidentified, and may, for convenience, be tentatively designated as factor M, as suggested by Blish and Sandstedt (1937).

Summary and Conclusions

Flours adjusted to the same sugar levels in baking will nevertheless show considerable differences in rate of *gas production*, especially in the later stages of fermentation. This is due to flour variations in quantity of a specific biocatalyst (activator) that stimulates maltose fermentation, baker's yeast being ordinarily highly deficient in its ability to ferment maltose in the absence of the activating factor, which, being as yet unidentified, is tentatively designated as "factor M."

Variations among different flours with respect to quantity of factor M make it very difficult to control *rate* of gas production, even though actual sugar levels may be fairly well adjusted to a standard basis. Therefore, the safest practical procedure in straight dough baking is to use a liberal quantity of sugar, and proof to standard height rather than to standard time.

Literature Cited

Blish, M. J., and Sandstedt, R. M.
1937 Biocatalytic activators specific for the yeast fermentation of maltose. J. Biol. Chem. **118**: 765–780.

Blish, M. J., Sandstedt, R. M., and Astleford, G. R.
1932 Sugars, diastatic activity, and "gassing power" in flour. Cereal Chem. **9**: 378–393.

Davis, C. F., and Worley, D. F.
1934 Correlation between diastatic activity and gassing power in commercial flours. Cereal Chem. **11**: 536–545.

Eva, W. J., Geddes, W. F., and Frisell, B.
1937 A comparative study of various methods of measuring flour gassing power. Cereal Chem. **14**: 458–480.

Genevois, L., and Pavloff, M.
1935 Fermentable sugars of wheat flour. Compt. Rend. Acad. **200**: 690–692.

Karacsonyi, L. P., and Bailey, C. H.
1930 Relation of overgrinding of flour to dough fermentation. Cereal Chem. **7**: 571–587.

Landis, Q., and Frey, C. N.
1936 The enzymes of flour in relation to yeast fermentation. Cereal Chem. **13**: 281–317.

Sandstedt, R. M., and Blish, M. J.
1934 Yeast variability and its control in flour gassing power tests. Cereal Chem. **11**: 368–383.

THE EFFECT OF VARIOUS TEMPERATURES AND TIME PERIODS ON THE PERCENTAGE OF GELATINATION OF WHEAT AND CORN STARCH AND OF CEREALS CONTAINING THOSE STARCHES

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Precise methods of starch and cereal cookery are lacking. To determine when starches are completely or desirably cooked has been and still is largely a matter of guesswork. Technical difficulties encountered in work with starch, the various methods used, and the multitude of variables involved doubtless explain, in part, the lack of experimentation which can be applied directly, in a practical way, to the cookery of starches and cereals. Approaches to the subject of starch cookery include: (1) gelatination tests, (2) digestibility tests, and (3) palatability tests.

By gelatination is meant the swelling of starch in contact with moisture. It has been studied chiefly by viscosity determinations but microscopic examination, changes in translucency, and iodocolorimetry tests have also been used.

Viscosity determinations have shown that as starch suspensions are heated in water, viscosity increases until a maximum value is reached. The source of the starch, its injury or modification in separating it from its natural source, the sizes of granules, the variation in starches of different horticultural varieties of the same species, the temperature of heating used, the rate of heating, the manipulation of the suspension during heating, and the length of time of heating have all been shown to affect viscosity readings.

Alsberg and Rask (1924) used viscosity tests with cooked starches in 3% to 10% concentrations and over a range of temperatures from 25° to above 90° C. The starches were so prepared that they were never subjected to the action of alkalies or other chemically active reagents. It was found that gelatination extends over a range of 25° to 30° C., reaching its maximum at 91° C. for cornstarch and 95° C. for wheat starch. The authors conclude that gelatination is not a sharp transition point like the melting points of crystalline substances but a gradual change which begins at a rather indefinite and variable

temperature and extends over a rather wide range of temperatures. Not until a temperature of 65° C. was reached did the starches used show appreciable swelling.

Various workers have observed that only in the presence of sufficient water does complete gelatination occur.

Woodruff and Nicoli (1931) used changes in translucency as a means of studying starch cookery. They used 5% concentrations of both root and cereal starches and observed the marked changes in translucency which occurred at temperatures which were quite specific for the different starches. The change in translucency did not occur sharply at one temperature for each starch but varied over a narrow range of one or two degrees C.

They cooled the starch solutions and examined the character of gels formed. All gels formed at the temperatures of maximum translucency were weak and showed syneresis. It was necessary to heat all suspensions to temperatures higher than those at which maximum translucency was observed in order to obtain gels of maximum strength. Gels formed from starch suspensions heated to 90°, 95°, and 99.5° C. were indistinguishable. Longer heating (15 to 30 min.) at the temperatures of maximum translucency did not yield gels as firm as those heated just to 90° C. or above and removed immediately from the source of heat. The cereal starches yielded firmer gels than the root starches. Some of the latter formed only viscous fluids.

Cunningham (1931) cooked cereal porridges by methods comparable to home cooking. He compared the blue solution color with iodine produced by the starch of the cooked cereals with the colors of standard solutions made with wheat starch. He assumed that a cereal porridge is cooked when 100% of its starch is gelatinized.

He found that the ratio of water to cereal is important in order to obtain 100% gelatination of the starch. He was able to obtain 100% gelatination only when at least 6 parts of water, by volume, to one part of granular cereal were used. Five minutes of boiling gave maximum gelatination. With a finer granulation two minutes of boiling was sufficient. He showed that with insufficient water, cooking proceeds for a given cereal to a point where the free water is absorbed. At this point gelatination stops and further cooking is useless.

The present study was undertaken as an attempt to obtain data which might serve as a basis for more definite recommendations than are now possible as to temperatures and time periods required for completely cooking cornstarch, wheat starch, cornmeal, and a granular wheat cereal made from the endosperm. A modification of Cunningham's method was used but boiling temperature, water-bath tempera-

tures of 85°, 90°, and 95° C., and a combination of boiling one or two minutes with additional cooking on the water bath were used.

Concentrations of 5% were used chiefly. A 5% starch concentration is suitable for starchy puddings, but a 5% concentration of cereal is somewhat thinner than one usually preferred as a breakfast food.

Because of the difficulty early encountered with cornmeal and the fact that time did not permit adequate experimenting with it, few data were obtained for cornmeal.

Both 1% and 5% concentrations of corn and wheat starch easily showed 100% gelatination when boiled one or two minutes. Percentages at temperatures below boiling are shown in Table I.

TABLE I

THE EFFECT OF VARIOUS TEMPERATURES AND TIME PERIODS ON THE GELATINATION OF CORN AND WHEAT STARCHES IN 5% CONCENTRATION

Temperature	Time of heating	Percentage of gelatination	
		Cornstarch	Wheat starch
Deg. C.	*Min.*		
95	5	95	95
	10	95	95
	15	95	95
90	5	90	90
	10	95	95
	15	95	95
85	5	70	50
	10	70	60
	15	70	60

The sample of cornmeal used was analyzed for starch by the Midwest Laboratories, Columbus, Ohio. The amount of cornmeal used for the tests was calculated to yield 5% of starch in the suspension. With boiling temperature only 50% gelatination was obtained with 2 minutes of boiling.

At 95° C. only 60% gelatination resulted from heating 5, 10, and 15 minutes. At 90° C., 10- and 15-minute heating periods gave only 50% gelatination, while at 85° C. only 30% gelatination resulted from 15 minutes of heating.

Granular wheat cereal made from the endosperm was used as cornmeal to furnish 5% of starch in the suspension. It showed much higher percentages of gelatination. Boiling a 5% suspension for 10 or 12 minutes gave 95% gelatination. Tables II, III, and IV give the results of heating a 5% concentration of granular wheat cereal for different time periods at temperatures below boiling or with a combination of boiling and water bath at 95° C.

TABLE II

THE EFFECT OF VARIOUS TEMPERATURES AND TIME PERIODS ON THE GELATINATION OF A 5% CONCENTRATION OF GRANULAR WHEAT CEREAL MADE FROM THE ENDOSPERM

Temperature	Time	Gelatination
Deg. C.	*Min.*	%
95	5	80
95	10	80
95	15	90
95	20	90
95	30	95
90	5	70
90	10	80
90	15	80
85	5	50
85	10	60
85	15	60

TABLE III

PERCENTAGES OF GELATINATION OBTAINED IN A 5% CONCENTRATION OF GRANULAR WHEAT CEREAL BY BOILING (ONE MINUTE) AND WATER-BATH HEATING AT 95° C. FOR VARIOUS TIME PERIODS

Temperature	Time	Gelatination
Deg. C.	*Min.*	%
95	5	80
95	10	85
95	15	95
95	20	95
95	30	95

TABLE IV

PERCENTAGES OF GELATINATION OBTAINED BY BOILING (TWO MINUTES) AND WATER-BATH HEATING AT 95° C. FOR VARIOUS TIME PERIODS

Temperature	Time	Gelatination
Deg. C.	*Min.*	%
95	3	85
95	5	90
95	10	95

Discussion

From the data it would appear that time, temperature, the presence of other substances, and size of particles are all factors affecting the rate and completeness of starch gelatination.

The starches were more rapidly and completely gelatinized at any given temperature or time period of heating than were the cereals containing those starches.

A temperature of 85° C. was not high enough to obtain a high percentage of gelatination. A temperature of 90° C. required appreciably longer than a temperature of 95° C. to bring about the same percentage of gelatination. By boiling for one or two minutes and later applying a temperature of 95° C. the rate of gelatination was noticeably increased. Boiling for two minutes as compared with one minute cut down the heating time at 95° C. 5 minutes to bring about 95% gelatination.

The maximum temperature obtainable in the top of a double boiler after the water in the lower compartment has boiled for about 15 minutes is about 95° or 96° C. The combination of boiling and water-bath heating used in the above experiments is comparable to that usually applied in cooking cereals in a double boiler in the home. For ease of combining cereal with water and to avoid lumping, the cereal is usually added gradually to boiling water, although there appears to be no proof that boiling temperature is necessary in cereal cookery. In the experiments performed in this study 95° C. brought about as high a percentage of gelatination as did boiling but required longer.

On the basis of Alsberg's findings 95° C. should be adequate for complete swelling of both corn and wheat starch but obviously all parts of the suspension should reach the necessary temperature.

Other factors than gelatination of starch which are involved in cereal cookery are the cooking of the protein fraction and the softening and hydration of the bran layer in whole and cracked cereals. Data are not adequate to prove to what extent protein and fiber interfere with gelatination or how much time allowance must be made to permit full gelatination of starch when protein and fiber are present. Alsberg and Griffing (1927) studied gluten coagulation and found no definite coagulation temperature. Only at 60° to 65° C. did they observe changes which indicate coagulation. Alteration of the gluten and decreased capacity for swelling in acid solution occurred over the range of temperatures from 50° to 80° C.

From most of our knowledge of protein cookery, it would seem that temperatures adequate for complete gelatination of the starch would be more than adequate to cook the protein.

Summary

High percentages of gelatination were obtained for corn and wheat starch and for a granular wheat cereal made from the endosperm.

The higher the temperature used, the shorter the period of heating required to obtain a high percentage of gelatination.

Both 1% and 5% concentrations of corn and wheat starch were 100% gelatinized in one or two minutes of boiling. When 5% concentrations of corn and wheat starch were cooked at a temperature of 95° C., which is comparable to the maximum temperature obtainable in the top of a double boiler, the heating period required to obtain 95% gelatination was increased to 5 minutes. This means, however, not 5 minutes of heating but 5 minutes after the temperature of the starch suspension reached 95° C. In the experiments recorded here, the time used to bring the suspensions to temperature was standardized to make comparisons more accurate.

Starch suspensions heated over water are best heated over boiling water since a temperature of 85° C. is not high enough to produce a very high percentage of gelatination. At the temperatures used, the sharpest rise in percentage of gelatination occurred within the range of 85° to 90° C.

Granular cereals, because of the presence of other substances or the size of particles, do not gelatinize as rapidly as do the corresponding starches. Boiling for one or two minutes before heating over water increases the rate of gelatination.

Greater uniformity of gelatination was observed with the two starches than with the two cereals containing those starches. No explanation is offered for the low percentages of gelatination obtained with cornmeal.

The experiments reported here support the general trend of opinion based on gelatination tests, digestion tests, and palatability tests that long cooking periods are not necessary at least for starches and granular cereals.

Literature Cited

Alsberg, C. L., and Griffing, E. P.
1927 The heat coagulation of gluten. Cereal Chem. **4**: 411–423.
Alsberg, C. L., and Rask, O. S.
1924 On the gelatinization of wheat and maize starch. Cereal Chem. **1**: 107–116.
Cunningham, N. T.
1931 The cooking of cereal porridges. Cereal Chem. **8**: 403–408.
Woodruff, S., and Nicoli, L.
1931 Starch gels. Cereal Chem. **8**: 243–251.

THE AMINO ACID DISTRIBUTION IN THE PROTEINS RESULTING FROM THE THERMAL FRACTIONATION OF WHEAT GLUTEN[1]

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The identity of the gluten proteins has been in doubt since wheat gluten has been fractionated into more than two components. The evidence indicates that wheat gluten is heterogeneous, containing at least one other fraction in addition to glutenin and gliadin.

The nature of the solvent has a marked effect on the properties of the wheat proteins. When alkali is used, time of exposure and concentration alter the glutenin in yield and composition. Therefore, neutral solvents have been recommended. Dill and Alsberg (1925) favored aqueous urea solution as the peptizing agent in glutenin preparation. Csonka and Jones (1927) reported the isolation of "α- and β-glutelins" from wheat glutenin. Gortner, Hoffman, and Sinclair (1929) found that no solution in a series of inorganic salts extracted a definite entity from various flours. The evidence of at least three gluten proteins was claimed by Blish and Sandstedt (1929).

In a study of the effect of heat treatment on flour, Geddes (1930) concluded that the identity of the gluten proteins was uncertain. Haugaard and Johnson (1930) fractionated gliadin into several components of varying physical properties by subjecting it in alcoholic solution to a series of low temperatures. Larmour and Sallans (1932) recommended the use of 0.007N acetic acid in glutenin preparation. Employing 20% urea solution as a peptizing agent, Cook and Alsberg (1931) prepared glutenin which showed greater uniformity than that prepared by other methods. Sandstedt and Blish (1933) obtained three fractions from wheat gluten, *viz.*, glutenin, mesonin, and gliadin, by thermal treatment of the gluten dispersed in 0.1N acetic acid and approximately 50% ethyl alcohol, saturated with sodium chloride.

Wheat gluten was reversibly fractionated by McCalla and Rose (1935) when the protein was dispersed in sodium salicylate solution and salted out by the addition of magnesium sulfate. A fairly complete

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analysis of wheat protein was described by Dakin (1918). By butyl alcohol extraction, the amino acids were separated by differences in their solubilities. In this work, β-hydroxyglutamic acid was first reported. Kingston and Schryver (1924) analyzed gelatin, using the "carbamate" method.

To separate the bases of a protein hydrolysate, Vickery and Leavenworth (1928) applied the technique of Kossel, which was based on the properties of their silver salts. This method has been quite successful. Town (1928) described the isolation of proline which he removed from a hydrolysate as its copper salt and finally isolated with Reinicke's reagent. Brazier (1930) hydrolyzed zein and separated several amino acids quantitatively by the formation of the metallic salts, chiefly those of copper. Applying Brazier's technique on glutenin, Damodaran (1931) analyzed glutenin and recommended the method since there was a satisfactory nitrogen recovery, and the nitrogen was identified in the form of individual amino acids.

Boyd (1933) studied the carbamino derivatives of the bases and dicarboxylic amino acids and commented favorably on the method used. A successful modification of the silver salts technique was described by Block (1934). Town (1936b) carried out a detailed analysis of gliadin in which the procedure was based on the properties of the copper salts of the amino acids. Many advantages of the method were pointed out. Glycine was estimated quantitatively with microtechnique by Patton (1935) using o-phthalic dialdehyde as the reagent to develop the characteristic color. Town (1936a) described another method for the quantitative determination of glycine by the use of nitranilic acid.

The purpose of this work has been to fractionate wheat gluten according to the method of Blish (1930) involving thermal treatment whereby three fractions, *viz.*, glutenin, mesonin, and gliadin, were obtained, and to compare the two fractions, glutenin and mesonin, on the basis of chemical composition. For analysis the method described by Brazier, dealing with the properties of the copper salts of the amino acids, has been followed, with some modifications that we have introduced.

Procedure

The wet crude gluten from 500 grams of Canadian hard spring second-patent flour was removed by washing with Dill and Alsberg solution (which was prepared by the addition of 4% NaH_2PO_4 solution to 4% Na_2HPO_4 solution to pH 6.8). The wet gluten weighed 275 g., of which 270 g. (equivalent of 184.41 g. dry gluten) was dispersed in 3.5 liters 0.1N acetic acid. About 7 g. of K_2SO_4 was stirred into the dis-

persion. After standing 24 hours with stirring at frequent intervals, 3.5 liters of 95% ethyl alcohol was added. On standing at 20°–21° C. glutenin settled out and was removed by centrifuging. The centrifugate was cooled and held at 12°–14° C., whereupon mesonin became insoluble. It was removed by decantation and finally by centrifugation. The gliadin precipitated out at 4° C. and from the supernatant liquid more gliadin was obtained by distilling off the alcohol and neutralizing the residue with sodium hydroxide. The protein preparations were dried *in vacuo* and the total nitrogen determined. The glutenin fraction contained 6.62% and the mesonin 13.73% nitrogen. Each sample was subjected to a complete analysis according to the method described by Brazier (1930) with certain modifications introduced which are detailed below.

Glutenin (4.5 g.) and mesonin (4.0 g.) were hydrolyzed with 20% sulfuric acid for 20 hours. The humin (1) [2] was filtered off and its nitrogen content determined. Barium hydroxide was added until the filtrate was only slightly acid. The precipitate was filtered off and washed and its nitrogen content determined. More barium hydroxide was added to the hydrolysate until it was slightly alkaline, and then warmed gently and a stream of air drawn through to remove the ammonia (2) which was collected in standard acid and determined by titration. The barium sulfate was filtered off and subjected to a Kjeldahl determination. The filtrate was concentrated to approximately 100 cc. and, still slightly alkaline, poured, while hot, into one volume of ethyl alcohol to precipitate the dicarboxylic acids. It was put in the refrigerator and after standing over night a second volume of alcohol was added. A third volume of alcohol was added the following day. The barium salts of the dicarboxylic acids settled out, were filtered off, and taken up with water. This aqueous solution was placed in an ice bath at 0° C. and saturated with HCl gas to obtain the ester of glutamic acid (3). In case the glutamic acid was incompletely isolated as its ester, the filtrate was neutralized by the addition of sodium hydroxide. To remove the sodium chloride, the solution was concentrated and about 4 volumes of ethyl alcohol were added. When submitted to a Kjeldahl nitrogen determination, the sodium chloride revealed only a trace of nitrogen. The filtrate, at its boiling point, was treated with copper carbonate to form the copper salts of glutamic and aspartic acids. Copper glutamate is water-soluble, while copper aspartate (4) is insoluble. The nitrogen content of each was then determined.

The remainder of the hydrolysate was neutralized quantitatively with dilute sulfuric acid and the precipitate of barium sulfate removed.

[2] These numerals refer to the corresponding entries in Table I.

The filtrate was concentrated and, at the boiling point, copper carbonate was added. It was allowed to cool and then filtered.

The water-insoluble copper salts "A" were taken up with dilute sulfuric acid. The excess copper carbonate, being insoluble, was removed. Copper was removed by treatment with hydrogen sulfide; the sulfide, after thorough washing, contained no nitrogen. The filtrate was treated at boiling temperature with freshly precipitated zinc hydroxide and boiled for thirty minutes. On cooling, the zinc salt of

TABLE I

NITROGEN DISTRIBUTION IN PRODUCTS OF SULFURIC ACID HYDROLYSIS OF THE GLUTENIN AND MESONIN FRACTIONS FROM WHEAT FLOUR GLUTEN

Total nitrogen	Glutenin 297.86 mg.		Mesonin 549.50 mg.	
	Mg. N	Percent of total nitrogen	Mg. N	Percent of total nitrogen
(1) Humin	13.31	4.47	6.90	1.26
(2) Ammonia	39.97	13.42	97.20	17.69
(3) Glutamic acid	18.86	6.33	83.36	15.17
(4) Aspartic acid	10.03	3.37	12.72	2.32
(5) Leucine	13.73	4.61	4.07	0.74
(6) Phenylalanine	20.46	6.87	10.38	1.89
(7) Lysine	5.41	1.82	———	———
(8) Histidine	3.94	1.32	6.62	1.20
(9) Arginine	29.01	9.74	38.19	6.95
(10) Alanine	35.42	11.89	56.85	10.35
(11) Proline	8.53	2.86	60.04	10.93
(12) Serine	1.93	0.65	21.09	3.84
(13) Hydroxyvaline	44.63	14.98	55.64	10.12
(14) Valine	3.58	1.20	1.21	0.22
(15) Bases	6.59	2.21	17.83	3.24
(16) Cystine	6.96	2.34	———	———
(17) Unidentified	12.82	4.30	9.47	1.72
a. Barium sulfate	10.75	3.6	20.97	3.82
b. Cadmium sulfide	———	———	9.85	1.79
c. Copper sulfide	7.04	2.36	6.31	1.15
Total nitrogen recovered	292.97	98.3	518.70	94.40

leucine (5) settled out, while that of phenylalanine (6) remained soluble. They were separated by filtration and the nitrogen content of each determined.

The water-soluble copper salts were concentrated almost to dryness and granulated with successive portions of acetone. The acetone extract from the glutenin hydrolysate, on evaporation, yielded a small gummy residue, water-insoluble, which, according to Town, is probably lysine (7). No N-containing acetone extract was obtained from the mesonin hydrolysate. The dried copper salts were extracted re-

peatedly with absolute methyl alcohol and thus were divided into two fractions: methyl-alcohol-insoluble "B" and methyl-alcohol-soluble "C."

Fraction "B" was taken up with water. Some material remained water-soluble and this portion was combined with "A." From the remainder of "B," the copper was removed as sulfide. Freshly precipitated zinc hydroxide was added to the filtrate, and when no more precipitate formed, mercuric chloride solution was added to precipitate the mercury salt of histidine (8), which was filtered off and its nitrogen content determined. The filtrate was freed from mercury and made acid with sulfuric acid to approximately 1%. Flavianic acid in 5% sulfuric acid was added and the mixture allowed to stand several days in the refrigerator. Arginine flavianate (9) crystallized out. The filtrate was concentrated and allowed to stand two days in the cold to remove a small quantity of arginine flavianate. The crystals were filtered off, dried *in vacuo* at 25°–30° C. and weighed. Arginine nitrogen was determined by digestion with potassium hydroxide, according to a Van Slyke procedure. The remainder of "B" contained alanine (10) and pyrollidone compounds. It was made to volume and the amino nitrogen content of it was ascertained by the Van Slyke method.

The methyl-alcohol-soluble portion "C" was distilled to remove the alcohol and the residue taken up with water. The copper was removed by treatment with hydrogen sulfide. According to Town, on addition of ethyl alcohol, tyrosine should precipitate out. This did not occur with either glutenin or mesonin. Cadmium chloride in 95% ethyl alcohol was added until no more precipitate formed. Mesonin did not yield a precipitate in this step, and so by treatment with hydrogen sulfide, the cadmium was removed from it and picric acid added, and the crystallization of proline picrate carried out as in the instance of glutenin.

The cadmium precipitate from glutenin was filtered off and dissolved in water. After removal of cadmium as the sulfide, picric acid was added. The solution was concentrated to about 20 cc. and refrigerated for two days. Crystals of proline picrate (11) formed. The crystals were removed and the mother liquor concentrated further, thus yielding more crystals of proline picrate. Both crystals and filtrates were extracted with ether several times. The crystals were recrystallized, dried *in vacuo*, and weighed. Ether extraction of the mother liquors gave unidentified fractions (17) from mesonin, a water-soluble extract, crystallizing to give crystals with a melting point of 117°–118° C. Glutenin yielded a gummy residue insoluble in water.

After ether extraction, the residue from the cadmium chloride

separation of the glutenin was combined with the cadmium chloride filtrate obtained in the separation of proline. The filtrate from the proline separation was made strongly acid with hydrochloric acid, and phosphotungstic acid was added to precipitate any bases that might be present. Both proteins showed the presence of a considerable quantity of bases (15) in this portion. The precipitate was removed by filtration through hardened filter paper, thoroughly washed with 1% HCl and taken up with water. Its total nitrogen and amino acid contents were ascertained. The bases of glutenin contained 36% non-amino nitrogen and those of mesonin, 65% non-amino nitrogen.

The filtrate from the cadmium-chloride-precipitated portion, according to Town, contained serine. The phosphotungstic acid was removed by precipitation with sodium hydroxide and then filtered. The filtrate was concentrated to about 50 cc. and three volumes of 95% ethyl alcohol were added. Serine (12) precipitated out readily and was removed by filtration. In aqueous solution, it was made to volume and total nitrogen and amino nitrogen determined. Glutenin revealed only a trace of serine, while mesonin contained 3.84% of its total nitrogen as serine.

The filtrate from the removal of serine was treated as in the method of Brazier. It was boiled with freshly precipitated zinc hydroxide for thirty minutes and filtered. The insoluble portion was the zinc salt of valine (14), and the soluble portion the zinc salt of hydroxyvaline (13). The amino and total nitrogen of hydroxyvaline were determined. The values for both proteins appear to be high, but it was found that of the total nitrogen in the fraction there was some non-amino nitrogen—in glutenin approximately 25% and in mesonin 60%, indicating the presence of at least one other nitrogen-containing compound in addition to hydroxyvaline.

Cystine (16) was estimated quantitatively by removing an aliquot of the neutralized hydrolysate and oxidation of its sulfur according to the method described by Morrow (1927). The solution was evaporated almost to dryness and fused with copper nitrate solution until all organic material was oxidized. The residue was taken up with a few cubic centimeters of distilled water and heated. Barium chloride in aqueous solution was added until no more precipitate formed. On being allowed to stand for several hours a precipitate of barium sulfate settled out and was filtered off, ignited, and weighed.

No cystine sulfur was obtained from mesonin. Glutenin gave a value of 15.9 mg. of sulfur as sulfate, the equivalent of 30.07 mg. of cystine.

Discussion

The proteins obtained by the fractionation of wheat gluten differed visibly, the glutenin being opaque and readily crumbled, while mesonin was fairly translucent and cohesive.

There were deviations from the method described by Brazier as applied to zein, and some modifications were required in this instance. No prolylphenylalanine was obtained which, according to Brazier, should appear in fraction "C." Also, tyrosine could not be isolated from glutenin and mesonin. The procedure was extended to separate serine as a result of the later work done by Town. In the isolation of proline, Town's procedure was followed, *i.e.*, by precipitation with cadmium chloride in alcohol. The unidentified portions were not found to have been described in the literature.

There was a heavy neutralization precipitate which had a strong tendency to adsorb nitrogen. Therefore it seemed advisable to remove the barium sulfate while the hydrolysate was still slightly acid. (On complete neutralization the remaining barium sulfate was more easily manipulated in the repeated washing necessary.) By this treatment considerable nitrogen loss was avoided. The amounts of humin nitrogen in glutenin (4.47%) and mesonin (1.26%) indicate chemical differences between the fractions.

The removal of ammonia was complete after about eight hours. Mesonin yielded 17.69% ammonia nitrogen; compared with 13.42% for glutenin, this indicates appreciable differences in constitution. Other values reported for glutenin range from approximately 12 to 16%. Mesonin ammonia nitrogen is higher than any recorded for the glutelins and lower than those of prolamins.

In the precipitation of the dicarboxylic acids, it was found (by previous experiment) that a more satisfactory separation took place when the hot solution, rather than cold, was treated with alcohol in three portions. This fraction was readily removed by filtration. It was thought that glutamic acid could be isolated effectively as the hydrochloride, but as such treatment gave a low yield the filtrate was neutralized quantitatively and the remaining glutamic acid, nearly 50%, separated from aspartic as its copper salt. It would therefore be more efficient to effect the separation of these two amino acids as their copper salts and not attempt to remove glutamic acid ester. The aspartic-acid content of glutenin (3.37%) is markedly higher than that of mesonin (2.32%), while for glutamic acid the two proteins showed even more striking differences, glutenin (6.33%) and mesonin (15.17%). The aspartic-acid values of glutenin are considerably higher than those reported by earlier workers, notably Damodaran, who studied glutenin

by this method, the value given by him being 1.85%. Since no tyrosine was obtained in this work, it seems possible that it has been included with the dicarboxylates and it is suggested that tyrosine should be readily isolated after removal of ammonia by concentrating the hydrolysate, rendering that amino acid insoluble.

It is important that the hydrolysate be neutralized quantitatively in order that an accurate separation of the copper salts may be executed since acidity or alkalinity changes their solubilities. In treatment of the copper salts, it is required that they be thoroughly dried. By evaporation of the mixture to a gummy residue, acetone extraction readily converted them into a fine powder. The acetone was added in rather small portions, stirred thoroughly, and filtered immediately. If allowed to stand there is a tendency for the copper salts to become resinous and present difficulty in subsequent extraction with alcohol. From glutenin, acetone extraction yielded a water-insoluble residue, soluble in ethyl alcohol. Mesonin failed to produce such a fraction. The nitrogen content of this extract was 1.82% of the glutenin nitrogen. Town assumed that this represents lysine.

Methyl alcohol extraction of the copper salts required the addition of several successive portions, each filtered off rapidly, until the final extract was almost colorless. The insoluble portion, when treated with water, showed some water-insoluble material which was combined with fraction "A."

Fraction "A" was treated according to Brazier's method. Significant differences were obtained between the two proteins in both leucine and phenylalanine, glutenin being very much higher in these than was mesonin. The latter revealed only a trace of leucine. (From 500 grams of wheat gluten Barnett isolated 10.8 g. of leucine, a lower quantity than obtained in this analysis.)

The copper sulfide required repeated extraction with hot water to remove adsorbed nitrogen; otherwise appreciable losses occurred.

The histidine values show small differences between the proteins studied and, compared with those formerly reported in glutenin, it is low. Some of it may be accounted for in the bases removed from fraction "C," as Town has stated that arginine shows a tendency to be distributed between "B" and "C," and histidine might show similar behavior. Removal of arginine as the flavianate presented no difficulty. Crystals formed on being allowed to remain in the refrigerator and were easily removed since the solubility of arginine flavianate in dilute sulfuric acid is low. Glutenin contained appreciably more arginine (9.74%) than did mesonin, indicating important differences between the two samples. Compared with other glutenin analyses, this value is a mean between the lowest and highest yields reported.

Brazier ascribed the remainder of fraction "B" to alanine. Her method, however, did not include the isolation of glycine. Town asserted that glycine and pyrollidone compounds remain in this portion. Since flavianic acid would contribute to the total nitrogen, the value in the table for alanine represents only the amino nitrogen and should be specified to include glycine and pyrollidone compounds. Assuming the presence of the latter, its nitrogen would not be included by the figure given and thus would represent some unrecovered nitrogen; a difference of 1.5% was obtained between glutenin and mesonin.

In the treatment of fraction "C," a modification suggested by Town was followed for removing proline by precipitation with alcoholic cadmium chloride. It is not clear why mesonin showed peculiar behavior in yielding no precipitate, for a good yield of proline picrate was finally obtained, markedly higher than that of glutenin. It should be remarked that cadmium sulfide requires repeated washing with hot water to free the precipitate of adsorbed nitrogen. All the proline of glutenin was removed in the cadmium-chloride precipitate. Its value obtained (2.86%) was considerably lower than the one reported by Damodaran (5.91%). Several recrystallizations were required for the isolation of proline from each protein.

Dissimilar unidentified material was obtained from glutenin and mesonin. That of the former was a water-insoluble residue which contained 4.30% of the total nitrogen. Of mesonin, the unidentified portion was water-soluble and formed crystals having a melting point 117°–118° C. and containing 1.72% of the total nitrogen of mesonin recovered as amino nitrogen.

The bases from "C" are probably a mixture of arginine, lysine, and histidine, as the results of the isolation of each one gave lower values than reported by other workers. The total recovery of bases in this work on glutenin (15.10%) is much greater than that reported by Damodaran (11.60%), who employed the silver salts technique. Analyses of glutelins have given basic nitrogen from approximately 19 to 27%. Comparison of the two samples in this detail revealed a notably higher basic nitrogen content in mesonin (21.74%) than in glutenin. Mesonin, according to its total basic nitrogen, resembles the glutelins and is unlike the prolamins previously studied as it has yielded a considerably higher basic nitrogen value.

The serine yield of glutenin is very low while that of mesonin (3.84%) is appreciable. (Removal of serine presented no difficulty.) The presence of non-amino nitrogen indicates, however, the presence of at least one other nitrogen-containing compound in this fraction which requires further investigation.

Hydroxyvaline and valine were separated according to Brazier's

method, *i.e.* the formation of their zinc salts. The valine recovery was low, as in mesonin there was actually only a trace of it, and in glutenin 1.20%. In this work the quantity of material used was too small to permit further identification of these amino acids, which seems desirable inasmuch as the values of hydroxyvaline are decidedly too high and a quantity of its nitrogen was found to be non-amino. (Damodaran reported that no hydroxyvaline was recovered in an analysis of glutenin, a remarkable contrast to that of 14.98% obtained in this work.)

Appreciable differences in nitrogen distribution have been observed between glutenin and mesonin in this work. Glutenin yielded significantly higher values for humin, leucine, valine, phenylalanine, arginine, alanine, aspartic acid, and hydroxyvaline than did mesonin and was lower in ammonia, glutamic acid, proline, serine, and total bases. Mesonin lacked cystine and yielded no acetone extract. The bases of glutenin and mesonin contained 64% and 35% amino nitrogen, respectively. It seems that mesonin resembles gliadin more than glutenin, notably because of its higher ammonia nitrogen and its higher dicarboxylic acid contents than glutenin. Assuming that the acetone extract is lysine, mesonin shows a lack of that amino acid, as does gliadin.

In the method of analysis described, there remains a quantity of nitrogen to be identified. The method has, however, been adequate in distinguishing between the two proteins studied and has rendered possible a more complete analysis than other procedures employing small quantities of material.

In view of the physical differences between the two proteins and those in their nitrogen distribution, the glutenin as here prepared differs chemically from mesonin, and evidence has thus been obtained contrary to that of the earlier view that wheat gluten is comprised of only two proteins, glutenin and gliadin.

Summary and Conclusions

Wheat gluten proteins may be fractionated by thermal treatment of the gluten dispersed in dilute acetic acid with 50% ethyl alcohol to give at least three fractions designated here as glutenin, mesonin and gliadin.

The visible characteristics indicate that the three fractions are dissimilar.

Chemical analysis of the sulfuric acid hydrolysate disclosed significant differences between the glutenin and mesonin fractions. Humin, leucine, valine, phenylalanine, arginine, alanine, aspartic acid, and hydroxyvaline were appreciably higher in the glutenin than in the

mesonin hydrolysate. Mesonin yielded more ammonia nitrogen, glutamic acid, proline, serine and total bases than did glutenin, while it lacked cystine and yielded no acetone extract.

The evidence obtained supports the view that wheat-flour gluten is constituted of more than two proteins.

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Literature Cited

Blish, M. J., and Sandstedt, R. M.
1929 The nature and identity of wheat glutenin. J. Biol. Chem. **85**: 195–205.
1930 The gluten and non-gluten proteins. Cereal Chem. **7**: 421–427.
Block, R. J.
1934 Determination of basic amino acids in small quantities of proteins by the silver precipitation method. J. Biol. Chem. **107**: 456–462.
Boyd, W. J.
1933 Derivatives of the monoamino-monocarboxylic acids. Biochem. J. **27**: 1838–1848.
Brazier, M. A. B.
1930 A new method for the separation of the products of protein hydrolysis. Biochem. J. **24**: 1188–1198.
Cook, W. H., and Alsberg, C. H.
1931 The preparation of glutenin in urea solution. Can. J. Res. **5**: 355–374.
Csonka, Frank A., and Jones, D. Breese
1927 Studies on glutelins. 1. The α- and β-glutelins of wheat. J. Biol. Chem. **73**: 321–329.
Dakin, Henry Drysdale
1918 On amino acids. Biochem. J. **12**: 290–317.
1920 Amino acids of gelatin. J. Biol. Chem. **44**: 499–519.
Damodaran, Maynath
1931 Amino acids of glutenin. Biochem. J. **25**: 190–199.
Dill, D. B., and Alsberg, C. L.
1925 Preparation, solubility and specific rotation of wheat gliadin. J. Biol. Chem. **65**: 279–304.
Geddes, W. F.
1930 Chemical and physico-chemical changes induced in wheat and wheat products by elevated temperatures. Can. J. Res. **2**: 65–90.
Gortner, R. A., Hoffman, W. F., and Sinclair, W. B.
1929 The peptization of wheat flour proteins by inorganic salt solutions. Cereal Chem. **6**: 1–17.
Haugaard, G., and Johnson, Arnold H.
1930 On the fractionation of wheat gliadin. Compt. Rendus. Trav. Lab. Carlsberg **18**: (No. 2), 1–138.
Kingston, Hilda L., and Schryver, Samuel
1924 Investigations on gelatin. III. The separation of the products of hydrolysis of gelatin by the carbamate method. Biochem. J. **18**: 1070–1078.
Larmour, R. K., and Sallans, H. R.
1932 A comparison of glutenin and gliadin prepared from one flour by various methods. Can. J. Res. **6**: 38–53.
McCalla, A. G., and Rose, J. T.
1935 Fractionation of gluten dispersed in sodium salicylate solutions. Can. J. Res. **12**: 346–356.
Morrow, Clarence Austin
1927 Biochemical Laboratory Methods. John Wiley & Sons, Inc., New York.

Patton, A. R.
1935 The determination of glycine in proteins. J. Biol. Chem. **108**: 267–272.
Sandstedt, R. M., and Blish, M. J.
1933 A new characterization of the gluten proteins. Cereal Chem. **10**: 359–366.
Town, Bernard William
1928 Isolation of pure l-proline. Biochem. J. **22**: 1083–1086.
1936a The micro-determination of glycine in protein hydrolysates. Biochem. J. **30**: 1833–1836.
1936b The separation of amino acids by means of their copper salts. Biochem. J. **30**: 1837–1844.
Vickery, H. B., and Leavenworth, V. S.
1928 Modifications of the method for the determination of the basic amino acids of proteins. The bases of edestin. J. Biol. Chem. **76**: 707–722.

REPORT OF THE 1937–38 COMMITTEE ON METHODS OF ANALYSIS[1]

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(Read at the Annual Meeting, May 1938)

The methods committee during the past year has conducted collaborative checks on the fifteen-minute moisture method and on two modifications of the ferricyanide maltose method. An attempt has been made to develop methods for the evaluation of malts and yeasts for bakery or mill use. A method has been developed for the more rapid determination of wheat and flour pigments.

Some progress has been made in the study of methods for the determination of proteolytic activity in flour. Dr. Hildebrand reports: "A series of flours was tested by the Sorenson formol titration method and by the copper hydroxide precipitation technique as described by Olsen and Bailey in 1925. The results by these two methods are in excellent agreement. Our calculations substantiate the statement made by Dr. Blish in 1918 that in the copper hydroxide precipitation procedure the nitrogenous materials determined are essentially in the peptide and amino acid class. Calculations show that the average chain length of the nitrogenous products is that of a di-peptide.

"In order to compare our results by the methods described above with those obtained with a method based on small changes in the structure of whole protein, we are attempting to determine the proteolytic activity of the same series of flour using the method recently proposed by Landis based on the change in gelatin rate of gelatin dispersions. Our results by this last procedure are not as yet complete. Accordingly we have no data to submit at this time. It is our intention to continue this work as we find time for it. We will be very glad to

[1] General report.

check any procedures which any member of the Association might have to suggest."

Recommendations

That the study of methods for testing yeast and the study of methods for the determination of protease activity in flour be continued.

That the methods developed for the evaluation of malts and the determination of wheat and flour pigments be studied collaboratively.

That further collaborative study be made of the maltose methods and the fifteen-minute moisture method.

That a study be made of the several methods for the determination of the neutralizing value of monocalcium phosphate.

That a method be developed for the evaluation of yeast foods based on gassing-power technique.

THE RAPID DETERMINATION OF MOISTURE[1]

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(Read at the Annual Meeting, May 1937)

In the constant endeavor to devise quicker and more convenient laboratory methods, especially for mill control purposes, many short-cuts have been suggested for the determination of moisture. Coleman and Dixon (1926) reported results obtained with a special type of drying oven which gave satisfactory results in 15 minutes. Davis (1935) reported that many cereal laboratories have shortened the time from the one hour required by the 130° method by the use of higher temperatures combined with special forced-air circulation. He concludes that the advantage of forced draft lies in the improved heat transfer and not in removal of vapor. Anderson (1936) showed that the time required to heat a sample of flour to oven temperature (100°) in the vacuum oven could be reduced from 1½ hours to less than 10 minutes if the sample was placed on a hollow brass plate through which steam was circulated.

From a consideration of these reports it would seem that the air oven is slow because of poor heat transfer to the sample and that, accordingly, it should be possible to accomplish a very great saving in time by making use of a heavy plate of a good heat conducting, high heat capacity metal, thus depending on the weight and high specific heat of the metal to heat the samples with no appreciable change in

[1] Sub-committee report, 1936–37 Committee on Methods of Analysis.

temperature of the plate. It would also seem possible to make use of such a metal plate for rapidly cooling the samples for weighing after removal from the oven. Aluminum plates were found to serve both purposes satisfactorily.

Method

A solid aluminum plate, ½-inch thick and of the proper dimensions to fit the oven, is placed on the lowest shelf brackets of the ordinary air oven. Another similar plate is kept at room temperature outside the oven. The temperature of the oven is adjusted to give a plate temperature of 140° C. The plate temperature may be conveniently determined by burying the bulb of the thermometer in a can of fine sand which rests directly on the plate (Bailey, 1914).

The uncovered aluminum moisture dish containing the two-gram charge of flour (evenly distributed over the bottom) is placed on the hot aluminum plate. The moisture-dish covers are left outside the oven while the samples are drying; this facilitates handling the hot samples after drying, for while the cover is cold one is able to transfer the hot sample from the oven with the bare hand. After fifteen minutes of drying, the cover is placed on the can while it is still on the hot place in the oven and then the can is transferred to the cold aluminum plate outside the oven. The samples are cooled on this plate for 2½ minutes—or until cold—and weighed. (Some types of moisture dishes will not cool in 2½ minutes, especially in hot weather.) No desiccator is necessary if the samples are not left more than about 5 minutes after becoming cold before weighing.

Table I shows the variation found in a series of moisture determina-

TABLE I

REPLICATE MOISTURE DETERMINATIONS ON FLOUR BY THE 130° ONE-HOUR METHOD AND THE 140° 15-MINUTE ALUMINUM PLATE METHOD

Position in oven	Moisture by	
	130° one-hour method	140° 15-minute
	%	%
Rear right corner	12.15	12.10
Rear left corner	12.14	12.14
Front right corner	11.75	12.02
Front left corner	11.81	12.04
Middle back side	12.05	12.09
Middle right side	12.08	12.11
Middle left side	12.02	12.05
Middle front side	11.77	12.07

tions on a flour, both by the 130° one-hour method and by the 140° 15-minute method. The samples were placed in various positions in the ovens so as to show the effect of position on the moisture obtained by the two methods. Bailey (1914) found a wide variation in temperature in different positions in the ordinary air oven. It is seen that in the ordinary air oven with the customary grid shelf (130° one-hour method) low moisture results are obtained if the samples are placed near the door. The aluminum plate used in the 140° 15-minute method seems to have eliminated the variation due to position. The moistures by the 15-minute method agree with those by the standard one-hour method.

In attempting to apply the 15-minute aluminum-plate method to ground wheat, it was found that low moistures were obtained. Table II gives the results of moisture determinations obtained on ground

TABLE II

REPLICATE MOISTURE DETERMINATIONS ON GROUND WHEAT BY THE 130° ONE-HOUR METHOD AND BY THE ALUMINUM-PLATE METHOD, HEATING FOR 15 MINUTES AND FOR 20 MINUTES

	Aluminum-plate method	
130° one-hour method	140°, 15 minutes	140°, 20 minutes
%	%	%
13.22	13.09	13.35
13.17	13.04	13.34
13.25	12.90	13.29
13.06	13.09	13.30

wheat by the 130° one-hour method as compared to the short-method heating for 15 minutes and for 20 minutes. These data show that it is necessary to heat for 20 minutes on the aluminum plate in order to obtain reliable moistures on more coarsely ground material such as ground wheat.

Literature Cited

Anderson, J. E.
1936 Some facts concerning vacuum-oven moisture determination. Cereal Chem. **13**: 437–452.
Bailey, L. H.
1914 Non-uniformity of drying oven temperatures. J. Ind. Eng. Chem. **6**: 585.
Coleman, D. A., and Dixon, H. B.
1926 A rapid moisture testing oven for cereal chemistry laboratories. Cereal Chem. **3**: 419–426.
Davis, C. F.
1935 Forced draft as an aid in air-oven moisture tests. Cereal Chem. **12**: 512–517.

COLLABORATIVE STUDY OF THE 15-MINUTE MOISTURE METHOD IN COMPARISON WITH THE OFFICIAL AIR-OVEN METHOD [1]

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(Read at the Annual Meeting, May 1938)

Results by the 15-minute heating method for the determination of moisture in flour proposed by Sandstedt in 1937 compare favorably with those by the Official A. O. A. C. Air-Oven Method. Replicate tests in the same laboratory have shown low variabilities and collaborative tests have been promising. The data thus far obtained are given herewith and further collaborative work is planned which may be extended to cereals other than wheat flour. Details of this method are printed elsewhere in this issue of Cereal Chemistry.[2]

Collaborators observed special precautions in handling, mixing, weighing, and drying the samples, to minimize all unnecessary error. Only four samples were dried at a time in the oven by each method and these were placed in the center of the shelf away from pilot lamp and door. The 140° C. temperature of the aluminum plate was measured by burying the thermometer in a small can of sand on the plate and with the bulb resting against the bottom of the can. The 130° C. temperature of the air oven was measured by a thermometer with the tip of the bulb level with the top of the dishes in the oven. Forced draft was not used. Each collaborator made duplicate tests on each sample and by both methods. Results on the three samples tested are tabulated below.

TABLE I

MOISTURE RESULTS OBTAINED BY COLLABORATORS ON WHEAT FLOUR SAMPLE NO. 1 BY 15-MINUTE METHOD AND BY OFFICIAL METHOD

Collaborators	1	2	3	4	5	6[2]
15-minute method (average 14.37)						
Subsample 1	14.64	14.55	14.00	14.41	14.37	14.39
Subsample 3	14.69	14.58	13.90	14.17	14.42	14.32
Official method (average 14.36)						
Subsample 2	14.64[1]	14.60	14.27	13.93	14.48	14.35
Subsample 4	14.65[1]	14.50	14.31	14.00	14.40	14.20

[1] Aluminum plate used in air oven.
[2] Collaborator used different ovens for each method.

[1] Sub-committee report, 1937–38 Committee on Methods of Analysis.
[2] R. M. Sandstedt, The rapid determination of moisture, Cereal Chemistry (this issue).

TABLE II

MOISTURE RESULTS OBTAINED BY COLLABORATORS ON WHEAT FLOUR SAMPLE NO. 2 BY 15-MINUTE METHOD AND BY OFFICIAL METHOD

Collaborators	1	2	3	4	5	6[2]
15-minute method (average 12.42)						
Subsample 1	12.50	12.50	12.30	12.48	12.43	12.40
Subsample 3	12.49	12.40	12.31	12.33	12.42	12.46
Official method (average 12.41)						
Subsample 2	12.62[1]	12.45	12.34	12.31	12.44	12.32
Subsample 4	12.57[1]	12.50	12.34	12.39	12.36	12.31

[1] Aluminum plate used in air oven.
[2] Collaborator used different ovens for each method.

TABLE III

MOISTURE RESULTS OBTAINED BY COLLABORATORS ON WHEAT FLOUR SAMPLE NO. 3 BY 15-MINUTE METHOD AND BY OFFICIAL METHOD[1]

Collaborators	1	2	3	4	5	6
15-minute method (average 14.43)						
Subsample 1	14.60	14.40	14.34	14.60	14.40	14.27
Subsa mple 3	14.56	14.25	14.26	14.55	14.41	14.24
Official method (average 14.41)[2]						
Subsample 2	14.67	14.45	14.44	14.44	14.41	14.18
Subsample 4	14.70	14.35	14.32	14.41	14.28	14.18

[1] Collaborators were asked to dry and cool all dishes just prior to weighing the samples and were asked to use only the regular oven grid shelf or wire shelf with the official method.
[2] Collaborators were asked to cool the dishes in the desiccator for ten minutes before reweighing.

The dimensions of the working space in the ovens of the different collaborators were similar. The dishes all had slip covers. Collaborator 4 had a cooling plate only 5 mm. thick and 25×38 cm. in area whereas the others had cooling plates 12 mm. or more in thickness and 25×30 cm. or less in area. Collaborator 2 had an oven shelf 6 mm. thick and generously perforated with holes 13 mm. in diameter. The rest had oven shelves 12 mm. or more in thickness. The dimensions of the moisture dishes used and the time allowed for cooling Samples 1 and 2 by the official method are shown in Table IV.

TABLE IV

DIMENSIONS OF MOISTURE DISHES USED IN ALL TESTS AND COOLING TIME ALLOWED FOR TESTS BY OFFICIAL METHOD ON SAMPLES NO. 1 AND NO. 2

Collaborators	1	2	3	4	5	6
Dish diameter (*mm.*)	50	52	40	50	51	50
Dish depth (*mm.*)	23	12	27	22	12	23
Cooling time (*min.*)	10	10	25	30	15	15

Discussion

The extent to which these differences in equipment and technique may have contributed to the differences in results shown in Tables I, II, and III is problematical, but the following should be pointed out:

Collaborators 3 and 4 obtained low results on Samples 1 and 2 by the official method, using a long cooling time in the desiccator. Their results, however, agreed with Collaborator 6. On Sample 3, when a short cooling time of 10 minutes was used by all, Collaborator 6 alone remained with low results.

In making tests by the 15-minute method, collaborators were asked to weigh the dried samples after 2½ minutes' cooling on the aluminum plate. Collaborator 4 reported that on Sample 2, Subsample 1, the dish felt slightly warm when weighed after 2½ minutes. Reweighed 2 minutes later when the dish was cool, the moisture result had dropped to 12.39%. Subsample 3, however, felt cold when weighed after 3½ minutes' cooling and showed 12.34% moisture when reweighed after a 5-minute total cooling on the plate. This collaborator experienced similar differences when testing Sample 3, but observed that after the dishes had once cooled until no longer warm to the hand the weight remained practically constant up to 10 minutes after the dish had been removed from the oven. Whether these results were peculiar to the thin cooling plate of large area used by this collaborator has not been determined; but for accurate results, dishes must attain room temperature before being weighed.

Collaborator 3 obtained lower results by the 15-minute method than by the official method on all samples. It is possible that the deep moisture dishes of smaller diameter than those used by the other collaborators may have contributed to this difference. Until this point is verified, dishes 50 mm. in diameter and not too deep should be used for the 15-minute method.

Acknowledgments

The assistance of the collaborators is gratefully acknowledged: A. A. Andre, C. F. Davis, J. M. Doty, Noel V. Knight, Grant W. Pearcy, R. M. Sandstedt, and J. Schumacher. This work was undertaken as a sub-project of the Committee on Methods of Analysis, A. A. C. C.

A COLLABORATIVE STUDY OF THE MODIFIED FERRICYANIDE AND CERIC SULFATE METHODS FOR DETERMINATION OF REDUCING SUGARS IN DIASTATIC ACTIVITY MEASUREMENTS[1]

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(Read at the Annual Meeting, May 1938)

The Methods of Analysis committee has undertaken a collaborative study of the modified ferricyanide procedure described by Sandstedt (1937) and the ceric sulfate method reported by Hildebrand and McClellan (1938). Briefly, in the ferricyanide procedure the reducing sugar formed by diastasis is oxidized by a known excess of standard alkaline potassium ferricyanide and the residual unreduced ferricyanide determined by titration with standard sodium thiosulfate. In the ceric sulfate technique the reducing sugar is oxidized by an excess of potassium ferricyanide and the ferrocyanide formed is titrated directly with standard ceric sulfate. An attempt has been made to secure adequate data for comparing these two methods as to average values obtained with different flours, variation of results between collaborators, and variation between replicate determinations.

Experimental

Four flour samples, which are described below in Table I, were taken:

TABLE I

DESCRIPTION OF FLOUR SAMPLES

Sample no.	Ash, %	Protein, %	Origin
1	0.40	11.20	S.W.
2	0.40	10.80	S.W.
3	0.38	10.30	S.W.
4	0.77	14.70	N.W.

In sample No. 1, the diastatic activity was artificially increased by the addition of 1% malted wheat flour. The sample was thoroughly mixed in a mechanically driven mixing device in order to secure as great homogeneity within the sample as possible. All other samples

[1] Paper No. 13, Journal Series, General Mills Research Laboratories. Sub-committee report, 1937–38 Committee on Methods of Analysis.

were untreated. Sub-samples were submitted to nine collaborators (C. F. Davis, R. M. Sandstedt, J. T. Flohil, A. D. Dickson, J. W. Evans, G. R. Astleford, R. H. Harris, W. G. Artis, and F. C. Hildebrand) with the following instructions:

Instructions

Digestion of flour samples should be carried out essentially according to the directions originally given by Blish and Sandstedt (1933).

10 g. of flour and 2 teaspoonfuls sand are to be introduced into a 250 c.c. Erlenmeyer flask. All ingredients should be warmed to 30° C. before mixing. 92 c.c. of M/10 acetate buffer of pH 4.6 should be added, the suspension shaken, and allowed to digest for exactly 60 minutes at 30° C. At the end of this time 4 c.c. of 10% sulfuric acid are added, the mixture shaken by rotation and 4 c.c. of sodium tungstate solution added and the suspension again mixed. Filter and discard the first 5 c.c. of filtrate. When approximately 50 c.c. has been collected, mix the filtrate thoroughly by shaking and proceed with the determination of reducing sugars as directed in the original description of the methods. (See Sandstedt (1937), and Hildebrand and McClellan (1938).)

Filter papers will be provided with the flour samples in order that no variation may be introduced because of differences in filtration.

With each method please conduct a blank determination, using in place of the sugar solution 5 c.c. of a mixture of buffer, sulfuric acid, and sodium tungstate solution in the usual amounts.

Please report three titrations for each flour sample with each procedure, giving in each instance milligrams of maltose per 10 g. of flour.

The results obtained by the various collaborators are given in Table II, and summarized and analyzed in Tables III, IV and V.

TABLE II

DATA SUBMITTED BY COLLABORATORS

(Values expressed as milligrams maltose per 10 grams flour)

Collaborator	N/10 Ferricyanide method				Ceric sulfate method				Mean both methods
	1	2	3	Mean	1	2	3	Mean	
				Sample no. 1					
A	426	426	426	426.0	395	400	407	400.7	413.4
B	432	432	432	432.0	404	419	424	415.7	423.8
C	444	444	450	446.0	442	441	440	441.0	443.5
D	442	443	443	442.7	420	422	435	426.7	434.7
E	395	395	395	395.0	394	395	395	394.7	394.9
F[1]	—	—	—	460.0	—	—	—	445.0	452.5
G	458	458	461	459.0	428	416	429	424.3	441.6
H	434	434	432	433.3	419	418	418	418.3	425.8
I	442	442	441	441.7	380	381	382	381.0	411.3
Mean, all collaborators				437.3				416.4	426.8

TABLE II—*Continued*

Collaborator	N/10 Ferricyanide method				Ceric sulfate method				Mean both methods
	1	2	3	Mean	1	2	3	Mean	
Sample no. 2									
A	222	229	227	226.0	233	236	239	236.0	231.0
B	235	239	236	236.7	257	259	258	258.0	247.3
C	259	257	255	257.0	254	255	256	255.0	256.0
D	245	244	244	244.3	235	237	236	236.0	240.2
E	234	234	237	235.0	238	237	238	237.7	236.4
F[1]	—	—	—	250.0	—	—	—	260.0	255.0
G	244	244	237	241.7	256	257	256	256.3	249.0
H	241	240	240	240.3	242	241	241	241.3	240.8
I	238	239	239	238.7	235	235	236	235.3	237.0
Mean, all collaborators				241.1				246.2	243.6
Sample no. 3									
A	118	126	123	122.3	140	141	141	140.7	131.5
B	130	130	130	130.0	154	155	154	154.3	142.2
C	139	139	139	139.0	155	156	154	155.0	147.0
D	139	139	139	139.0	153	155	154	154.0	146.5
E	145	142	145	144.0	143	144	145	144.0	144.0
F[1]	—	—	—	142.0	—	—	—	145.0	143.5
G	116	114	116	115.3	135	137	138	136.7	126.0
H	135	137	137	136.3	149	151	149	149.7	143.0
I	132	133	133	132.7	150	154	151	151.7	142.2
Mean, all collaborators				133.4				147.9	140.7
Sample no. 4									
A	276	279	279	278.0	273	279	279	277.0	277.5
B	280	283	280	281.0	316	321	322	319.7	300.4
C	296	296	296	296.0	297	299	299	298.3	297.1
D	291	294	293	292.7	290	297	293	293.3	293.0
E	279	279	276	278.0	277	276	276	276.3	277.2
F[1]	—	—	—	293.0	—	—	—	291.0	292.0
G	298	295	295	296.0	296	293	298	295.7	295.8
H	290	288	287	288.3	293	295	294	294.0	291.1
I	283	284	284	283.7	295	295	296	295.3	289.5
Mean, all collaborators				287.4				293.4	290.4

Collaborator	All Flours									
	A	B	C	D	E	F	G	H	I	All
N/10 ferricyanide	263.1	269.9	284.5	279.7	263.0	286.3	278.0	274.5	274.2	274.8
Ceric sulfate	263.6	286.9	287.3	277.5	263.2	285.3	278.3	275.8	265.8	276.0
Both methods	263.4	278.4	285.8	278.6	263.1	285.8	278.2	275.1	270.0	275.4

[1] Collaborator F reported only mean values.

TABLE III

SUMMARY OF COLLABORATIVE DATA

(Values expressed as milligrams maltose per 10 grams flour)

Sample no.	Mean	Standard error between collaborators	Coefficient of variation, %	Standard deviation of triplicates
		N/10 Ferricyanide Method		
1	437.3	19.59	4.48	1.22
2	241.1	8.90	3.69	1.90
3	133.4	9.49	7.11	1.39
4	287.4	7.41	2.58	1.22
Mean, all samples	274.8	11.35	4.47	1.43
		Ceric Sulfate Method		
1	416.4	22.07	5.30	4.99
2	246.2	10.80	4.39	1.08
3	147.9	6.59	4.46	1.01
4	293.4	12.71	4.33	1.96
Mean, all samples	276.0	13.04	4.62	2.26
		Mean, Both Methods		
1	426.8	20.83	4.89	3.10
2	243.6	9.85	4.04	1.49
3	140.7	8.04	5.78	1.20
4	290.4	10.06	3.46	1.59
Mean, all samples	275.4	12.20	4.54	1.84

TABLE IV

ANALYSIS OF VARIANCE OF COLLABORATIVE DATA

Variation due to:	D.F.	Sum of squares	Mean square	F	5% point
		N/10 Ferricyanide Method			
Flours	3	1,131,241	377,080	1053.3	3.07
Collaborators	7	4,698	671	1.874	2.49
Flours × collaborators	21	7,527	358 (error)	—	—
Residual error	64	196	3.06	—	—
Total	95	1,143,662	—	—	—
		Ceric Sulfate Method			
Flours	3	871,206	290,402	882.7	3.07
Collaborators	7	7,973	1,139	3.462	2.49
Flours × collaborators	21	6,906	329 (error)	—	—
Residual error	64	670	10.47	—	—
Total	95	886,755	—	—	—

TABLE IV—*Continued*

Both Methods					
Flours	3	1,992,972	664,324	2565.0	3.07
Collaborators	7	10,771	1,539	5.942	2.49
Methods	1	95	95	2.726	248
Flours × collaborators	21	9,000	429	1.656	2.09
Flours × methods	3	9,475	3,158	12.193	3.07
Collaborators × methods	7	1,900	271	1.046	2.49
Fl. × collab. × methods	21	5,433	259 (error)	—	—
Residual error	128	866	6.766	—	—
Total	191	2,030,512	—	—	—

(Values of Collaborator F not included.)

TABLE V

ANALYSIS OF VARIANCE OF INDIVIDUAL SAMPLE DATA

Variation	D.F.	Sum of squares	Mean square	F	5% point
Sample no. 1					
Between methods	1	1,968	1,968	4.74	4.49
Within methods	16	6,639	414.9	—	—
Total	17	8,607	—	—	—
Sample no. 2					
Between methods	1	117	117.0	1.20	4.49
Within methods	16	1,566	97.89		
Total	17	1,683			
Sample no. 3					
Between methods	1	945	945.0	14.2	4.49
Within methods	16	1,068	66.75	—	—
Total	17	2,013	—	—	—
Sample no. 4					
Between methods	1	165	165.0	1.53	4.49
Within methods	16	1,728	108.0	—	—
Total	17	1,893	—	—	—

Discussion of Results

In the summary and analysis of the data shown in Tables III and IV, certain points are of particular interest. Considering the four samples as a whole, it is evident that there is a significant variation,

among the several collaborators, in the relative differentiation between flours by the two methods, and in the variation among triplicate determinations.

The differences among collaborators are not at all surprising, in view of the many potential sources of error inherent in such measurements of diastatic activity. These variations, however, are apparently to be ascribed to errors occurring prior to the actual sugar determination, since each collaborator obtained essentially the same results with either method, as shown by the non-significance of the variance for the interaction of flours × collaborators in Table IV. This may also be seen from the average values for all flours given in Table II, which indicate that with only two exceptions the results obtained with the two methods are in excellent agreement.

The collaborators as a whole obtained better agreement between triplicate determinations with the ferricyanide method. It is interesting to note, however, that this larger experimental error in the instance of the ceric sulfate method is confined to sample No. 1, as is shown in Table III. It is of further interest to note that in this case four collaborators obtained widely divergent values, and four report results showing excellent agreement between triplicates. Lacking more detailed information, it is difficult to explain this peculiarity.

The two methods give significantly different results in differentiating between flours. It seemed of interest to investigate further the source of this variation. Individual variance analyses were made for each flour, as shown in Table V. These computations show that by far the largest portion of the variation is to be found in the results with sample No. 3. With Nos. 2 and 4, the difference between methods is not significant, and with flour No. 1 the difference has only slight significance.

This study confirms the findings of C. F. Davis (1937) that variation between collaborators is highest in the case of highly active, malt-treated flours. That this is not due to lack of homogeneity within the sample is indicated by the calculation of the coefficient of variation between collaborators shown in Table III. The value for sample No. 1 is not far from the average value for all flours.

Various collaborators were evenly divided as to preference between the two methods. One worker stated that he had been using both methods side by side in routine analysis for several months and considered the results equally reliable.

The author wishes to acknowledge his indebtedness to the collaborators who participated in this study, and to Dr. Forrest Immer, who made the variance analysis given in Table IV.

Summary

The modified ferricyanide and ceric sulfate procedures gave similar results when employed in the determination of reducing sugars in a series of collaborative diastatic activity measurements. With a flour of relatively low diastatic activity, the ferricyanide procedure gave significantly lower values, showed higher variability between collaborators and slightly greater experimental error. With two flours of medium diastatic activity, there was no difference evident between methods. With a flour of relatively high diastatic activity, the ceric sulfate method gave slightly lower results and showed somewhat larger variation between collaborators and higher experimental error.

On the basis of this study, there is no evidence of distinct superiority of one method over the other, and the relative acceptability of either method may well be determined by individual preference as to convenience and ease of operation.

Literature Cited

Blish, M. J. and Sandstedt, R. M.
1933 An improved method for the estimation of flour diastatic value. Cereal Chem. **10**: 189–202.

Davis, C. F.
1937 A collaborative study of the Blish-Sandstedt, Schoorl, and Bertrand methods for determining reducing sugars in flour diastatic activity measurements. Cereal Chem. **14**: 74–85.

Hildebrand, F. C. and McClellan, B. A.
1938 An improved method of sugar determination in diastatic activity measurements. Cereal Chem. **15**: 107–113.

Sandstedt, R. M.
1937 The adaptation of the ferricyanide maltose method to high diastatic flours. Cereal Chem. **14**: 603–604.

A COMPARISON OF METHODS FOR EVALUATING THE DIASTATIC PROPERTIES OF MALT AS A FLOUR SUPPLEMENT[1]

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(Read at the Annual Meeting, May 1938)

In the evaluation of the diastatic properties of malt products, use has generally been made of the Lintner determination or some of its modifications with soluble or gelatinized starch used as a substrate. The malt treatment of diastatically deficient flours has brought about the need of more efficient methods especially adapted to this type of control. Leatherock, McGhee, and Giertz (1937) compared various malt flours as flour supplements by adding definite amounts of the different malts to a standard raw flour as a substrate and made comparisons of the Rumsey autolytic maltose values as determined by the Blish and Sandstedt (1933) procedure. For practical purposes this method was found to be satisfactory because of the high correlation that usually exists between the autolytic maltose values and the dough gas-production values which are the baker's index of the diastatic ability of the flour.

This study is a comparison of the Rumsey autolytic maltose values and pressure-meter gas-production values (as determined on flours treated with different malt products) and the Lintner value of the malts.

Samples

In Table I is a description of the fifteen malt samples used in this study. These malts from various grain sources and different manufacturers are representative of diastatic products used to supplement flour in either the milling-process of bake-shop production.

Two flour substrates were selected which were somewhat different in their response to the action of malt. Flour A was rather soft in character and apparently milled to a relatively fine granulation. Flour B was a standard baker's patent milled from 1937 Kansas Tenmarq wheat which had baking characteristics very similar to those of spring-wheat flour. This flour was a granular type.

[1] Sub-committee report, 1937–38 Committee on Methods of Analysis.

	Flour A	*Flour B*
Protein % (15% moisture basis)	12.25	12.20
Ash % (15% moisture basis)	0.43	0.44
Maltose value	240 mgm.	214 mgm.
Pressure-meter value	401 mm.	312 mm.

TABLE I

A DESCRIPTION OF THE DIASTATIC MALT PREPARATIONS USED AS FLOUR SUPPLEMENTS

Malt no.

1. 1935 malted barley, milled Dec., 1937, to 67.5% flour.
2. 1936 malted barley, milled Dec., 1937, to 66.1% flour.
3. 1936 malted wheat, milled Dec., 1937, to 70.8% flour.
4. 1937 malted wheat, milled Dec., 1937, to 71.0% flour.
5. Malted wheat-flour samples submitted as representing stages of the diastatic development in malt.
6. Malted wheat-flour samples submitted as representing stages of the diastatic development in malt.
7. Malted wheat-flour samples submitted as representing stages of the diastatic development in malt.
8. Malted wheat-flour samples submitted as representing stages of the diastatic development in malt.
9. Submitted sample of malted wheat flour.
10. Submitted sample of malted rye, milled Dec., 1937, to 66% flour
11. Submitted sample spray dried malt, 180° L.
12. Submitted sample malt syrup, low diastatic.
13. Submitted sample malt syrup.
14. Malted wheat flour submitted as a bad sample. Sample carried a strong musty odor.
15. Submitted sample malted wheat flour.

Procedure

Each flour substrate was treated with increments of malt amounting to 0.0625%, 0.125%, and 0.25% or more. At each treatment maltose tests were conducted in duplicate according to the method of Blish and Sandstedt (1933) and gas-production tests were carried out in duplicate in the pressure meter for 6 hours with 3% yeast as described by Sandstedt and Blish (1934). The average values of these duplicate tests were plotted against the increasing amounts of each malt added as illustrated in Figure 1 with five different malts and substrate B.

All malt samples were submitted to the 1937–38 A. A. C. C. committee on Methods of Malt Analysis for Lintner Determination. These tests were conducted in the Grain Research Laboratories of the U.S.D.A. Bureau of Agricultural Economics under the direction of Dr. D. A. Coleman.

Discussion

For the purpose of making a comparative evaluation of the malts it would seem logical to determine the amount of each malt that is required to raise the maltose value (or the gassing-power value) of the flour to a certain predetermined figure, preferably a value which corresponds to commercial practice. Then the value of each malt will be the quantity inverted; that is, 1/quantity = value. These values

can then be compared on a percentage basis by giving the standard malt a value of 100. Consideration should also be given to values that give best differentiation between the malts.

Treatment levels for the two substrates in this study were selected independently of each other because of the difference in malt response of these substrates. In Table II it may be observed that the response in substrate B was much greater than the response in substrate A. This indicates the necessity of using one flour as a substrate in making comparisons of any particular group of malt samples. In this group of samples malt No. 3 was arbitrarily taken as a standard of 100%

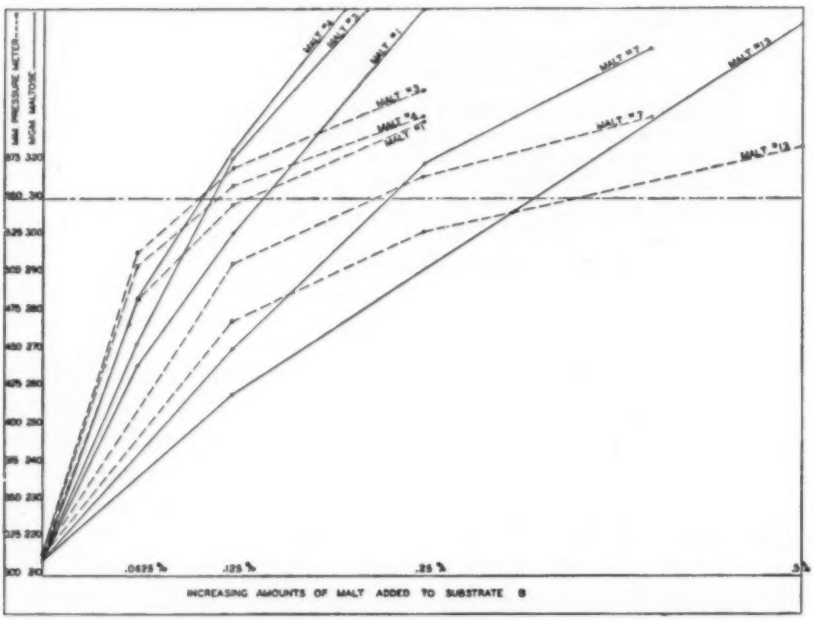

Fig. 1. Relation of maltose and total gas production (pressure-meter) values with increasing amounts of malts added to substrate B.

value for comparison and the value of the other malts was related to this malt on a percentage basis.

Table II shows that evaluations by the ferricyanide method and by the gassing-power method do not have the same relation to each other on all malts. With malts No. 2 and No. 4 there was a definite tendency for the maltose evaluation to be somewhat higher than the gas-production figures would indicate from a comparison with the results obtained with the other malts. From Figure 1 it may be observed that with malts such as No. 4 the gassing power increases more slowly with increase in malt than is true with some other malts

and that above certain malt treatment the maltose-value method gives the malt an abnormally high value when compared to the value obtained by the gassing-power method. The tendency for these malts to be rated in a similar manner on both substrates (Table II) is indicated by a + 0.989 correlation between the maltose evaluations and a + 0.983 correlation between the pressure-meter evaluations. These high correlations indicate the general reliability of the use of a raw flour substrate as a basis for malt comparison.

TABLE II

THE AMOUNT OF VARIOUS MALTS REQUIRED TO RAISE THE TEST VALUES TO ARBITRARILY CHOSEN LEVELS FOR TWO FLOUR SUBSTRATES

Malt no.	Substrate A				Substrate B			
	Amount of malt to raise to				Amount of malt to raise to			
	320 mgm. maltose		520 mm. Hg		310 mgm. maltose		550 mm. Hg	
	% (A_m)	% of standard malt	% (A_p)	% of standard malt	% (B_m)	% of standard malt	% (B_p)	% of standard malt
1.	0.165	75.8	0.167	73.6	0.144	76.4	0.142	72.5
2.	0.111	112.6	0.135	91.1	0.086	127.9	0.107	96.3
3.	0.125	100.0	0.123	100.0	0.110	100.0	0.103	100.0
4.	0.123	101.6	0.159	77.4	0.104	105.7	0.137	75.2
5.	0.148	84.5	0.141	87.2	0.134	82.1	0.115	89.6
6.	0.225	55.5	0.180	68.3	0.204	53.9	0.166	62.0
7.	0.298	41.9	0.243	50.6	0.227	48.4	0.221	46.7
8.	1.560	8.0	1.440	8.5	1.540	7.1	1.425	6.7
9.	0.334	37.5	0.284	43.4	0.287	38.3	0.250	41.2
10.	0.141	88.6	0.125	98.4	0.131	84.0	0.120	85.8
11.	0.144	86.8	0.164	75.0	0.125	88.0	0.152	67.8
12.	0.501	24.9	0.504	24.4	0.499	22.0	0.501	20.6
13.	0.335	37.3	0.337	36.5	0.320	34.4	0.344	29.9
14.	0.317	39.4	0.337	36.5	0.235	46.8	0.275	37.5
15.	0.205	61.0	0.195	63.1	0.157	70.0	0.177	58.2
					$rA_mB_m = +0.989$		$rA_pB_p = +0.983$	

Table III shows that there is a correlation of + 0.96 between the evaluations by the pressure-meter method and by the maltose-value method. The lowest correlation between different methods is between the Lintner values and the pressure-meter evaluation, where the correlation is + 0.87. This variable reaction to different tests would indicate that the conditions of the test should be as near as possible to the conditions of actual use of the product. On the basis of this theory the pressure-meter test would give the more reliable results and the relatively high evaluation placed on malts No. 2 and No. 4 by

the maltose test is perhaps an example of unreliable valuation of the malts.

The baking test, which is the final measure of the suitability of malt as a flour supplement, found these malts generally in line with the test values established by the maltose and pressure-meter methods but the complexity of factors involved did not permit exact measurements of factors with reference to the diastatic properties under study. Baking tests with no added sugar, with 3% yeast, and with 3 and 4 hours' fermentation of samples treated with malts No. 1, No. 2, No. 3, and No. 4, showed no significant differences when both substrates were treated to give the same maltose level or the same gas-

TABLE III

A Comparison of the Average Percent of Malt Supplements on Flour Substrates A and B with the Lintner Evaluation

Malt no.	Average percent rating on Substrates A and B		Degrees Lintner	Degrees L. as percent of standard malt
	Maltose	Pressure-meter		
	x	y		z
1.	76.1	73.0	121.1	122.2
2.	120.2	93.7	128.1	129.3
3.	100.0	100.0	99.1	100.0
4.	103.6	76.3	103.0	103.1
5.	83.3	88.4	92.0	92.8
6.	54.7	65.1	71.3	71.9
7.	45.1	48.6	61.4	61.9
8.	7.5	7.6	39.2	39.5
9.	37.9	42.3	127.1	128.2
10.	86.3	92.1	115.8	116.7
11.	87.4	71.4	124.4	125.5
12.	23.4	22.5	24.0	24.2
13.	35.8	33.2	57.0	57.5
14.	43.1	37.0	71.2	71.8
15.	65.5	60.6	100.0	100.9
	$r_{xy}=+0.96$	$r_{xz}=+0.89$	$r_{yz}=+0.87$	

production level. This would suggest that for practical purposes the discrepancy between the two methods should not be regarded as having great importance.

Three months after the original data in Table II were completed the work was repeated with samples 1, 2, 3, and 4. The malt flours and substrate flours had been stored under ordinary warehouse conditions (unheated) for the months of January, February, and March. For both substrates and with all four malts, from 10% to 30% more malt was required to treat to the original levels chosen. No work was done to determine the source of this difference, which could have been caused by a change in the susceptibility of the substrate or in the diastatic

strength of the malt flour. It was of interest, however, that with malt No. 1 a significantly greater amount of malt was required to raise the pressure-meter values to the established level than was required to raise the maltose values to the established level. With malt No. 2 the reverse of this condition was true. This could indicate that all factors in the malt flour affecting the different test values did not depreciate at the same rate.

Summary and Conclusions

In a study of the valuation of fifteen various diastatic malt products to be used as flour supplements, three methods of analysis were used: (x) the Rumsey autolytic maltose determination and (y) gas-production determinations, on malt treated flours, and (z) the Lintner determination on the malt. Correlations of evaluations by the three methods were: $r_{xy} = +0.96$, $r_{xz} = +0.89$, and $r_{yz} = +0.87$. The necessity and reliability of using one flour as a substrate in making comparative evaluation of different malt samples was pointed out and also the fact that with some malts the autolytic maltose values might be in excess of the gas-production index; however, for practical purposes at the levels to which flours are generally treated this discrepancy appeared to have no great significance.

Because of the difference in the responses of various malts in a raw-flour substrate at different levels of treatment it appears that in making close comparisons of the quantity of malt necessary to obtain a definite diastatic or gassing value in flour at least two treatments should be made near the chosen level and the proper amount ascertained from a graph.

Literature Cited

Blish, M. J., and Sandstedt, R. M.
1933 An improved method for estimation of flour diastatic activity value. Cereal Chem. **10**: 189–202.

Leatherock, L. E., McGhee, H. W., and Giertz, J. W.
1937 Diastatic activity in connection with flour mill control. Cereal Chem. **14**: 161–181.

Sandstedt, R. M., and Blish, M. J.
1934 Yeast variability and its control in flour gassing power tests. Cereal Chem. **11**: 368–383.

THE RELATION OF ALPHA- AND BETA-AMYLASES TO THE EVALUATION OF MALTS IN FLOUR TECHNOLOGY [1]

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The discrepancies which Davis and Tremain (1938) found between the Lintner values and values obtained by methods involving flour as the substrate in the series of malts which they used suggest differences in the predominance of certain amylases in the malts. Consequently it was considered of interest to investigate these malts in respect to their amylase content and to attempt to determine which of the amylases is of greatest importance in malts used as flour supplements.

It was also considered desirable to test the reliability and general applicability of Davis and Tremain's (1938) malt evaluations when these malts were used with an entirely different flour as substrate. The equivalent quantities of the malts, calculated from their pressure-meter evaluations, were added to samples of an undiastated flour. Gassing powers were determined by pressure meter on this series of malt treatments, all samples supposedly diastated to give equal gassing powers. The results are shown in Table I.

TABLE I

PRESSURE-METER GASSING-POWER DETERMINATIONS ON FLOUR DIASTATED WITH EQUIVALENT QUANTITIES OF MALT

Malt no.	Quantity added to flour	4-hour gassing power	6-hour gassing power
	%	*mm. Hg*	*mm. Hg*
0	0	190	202
1	0.151	334	378
2	0.114	341	385
3	0.110	335	381
4	0.145	351	395
5	0.125	332	378
6	0.170	335	382
7	0.225	332	380
8	1.450	335	384
9	0.262	338	385
10	0.127	343	390
11	0.155	338	381
12	0.480	329	369
13	0.333	328	368
14	0.297	340	385
15	0.180	342	392

[1] Sub-committee report, 1937–38 Committee on Methods of Analysis.

These data offer a very strong confirmation of the reliability and applicability of this method of evaluating malts. The gassing powers are remarkably consistent. Malt 4 gave rather high results, indicating that its rating for this flour was somewhat low. The malt syrups (Malts 12 and 13) gave low results, which are considered to be due to deterioration from standing at room temperature for the four months between Davis and Tremain's original evaluations and this series of tests.

It also seemed advisable to check the action of these malts on boiled potato starch in order to confirm the information which the Lintner values convey. For this purpose, 10 mg. of malt was allowed to act for one hour on 50 c.c. of 1% boiled potato starch buffered at pH 4.7. The sugar produced was determined by the ferricyanide method. These data were then recalculated on a percentage basis with the value obtained on Malt 3 as the standard (100) so as to make them comparable to the other data presented. (All data given in Table II were

TABLE II

COMPARISON OF DIFFERENT METHODS OF EVALUATING MALTS

Malt no.	Davis and Tremain evaluations on basis of			Evaluations based on	
	Gassing power	Maltose value	Lintner value	Alpha-amylase content	Action on boiled potato starch
3	100	100	100	100	100
2	94	120	129	131	159
5	88	83	93	90	95
10	87	86	117	95	98
4	76	104	103	110	101
1	73	76	122	90	134
11	71	87	126	81	98
6	65	55	72	76	89
15	61	63	101	85	100
7	49	45	62	65	85
9	42	38	128	81	114
14	37	43	72	59	62
13	33	36	58	38	52
12	23	23	24	17	22
8	8	8	40	23	63

so compiled.) From Table II it is seen that measuring the action of the malts on boiled potato starch gives the same information and the values are similar to the Lintner values calculated on the same percentage basis.

Alpha-amylase was determined by a modified Wohlgemuth method: The time was determined for the disappearance of the blue starch-iodine color when 50 mg. of malt was acting on 25 c.c. of 2% soluble starch buffered at pH 4.7. These values, also calculated on a percentage basis with Malt 3 as the standard, are given in Table II.

There is a very high correlation between the alpha-amylase content and the values which Davis and Tremain obtained by using these malts on flour as a substrate, the best correlation being with his evaluation based on maltose values. It appears that alpha-amylase is the most important factor determining the values of malts as flour supplements. It is interesting that Munz and Bailey (1937) concluded that the degradation of dough plasticity or increase in mobility appears to be affected by alpha-amylase contributed by malted wheat flour.

Occasionally some other factor disturbs the correlation of alpha-amylase with gassing power. Malts 2 and 4 are illustrative of malts high in alpha-amylase but not correspondingly able to confer high gassing powers to flours. This other factor might possibly be the enzyme responsible for raw starch saccharification. Whatever this factor may be, it registers in the pressure-meter evaluation but does not in other methods.

The correlation of alpha-amylase with Lintner value is also high, giving evidence that alpha-amylase is also a major factor in the evaluation of malts by the Lintner procedure. But high Lintner values may also be due to high beta-amylase; accordingly it is of interest to note that Malts 1, 9, 10, 11, and 15 have much higher Lintner values than would be expected from their alpha-amylase content and also that these malts do not have a corresponding ability to increase the gassing power of flour.

All flours contain an abundance of beta-amylase—so much so that increasing the beta-amylase in a flour to three times its original amount by the addition of the purified enzyme causes no increase in either the maltose or gassing-power values. With such an excess of beta-amylase already present in the flour, the beta-amylase content of a malt supplement would not be expected to be of any consequence. Accordingly malts which have high Lintner values due to high beta-amylase (Malts 1, 9, 10, 11, and 15) will not be correspondingly active on flour.

Summary and Conclusions

The method of evaluating malts by pressure meter as outlined by Davis and Tremain (1938) promises to be reliable and useful.

Alpha-amylase is the amylase of predominant importance in malts which are used as flour supplements.

Because of the universally high beta-amylase content of flours themselves, the beta-amylase content of a malt is of no consequence. Accordingly the Lintner value may not correspond to the true value for baking purposes.

Literature Cited

Davis, C. F., and Tremain, H. E.
1938 A comparison of methods for evaluating the diastatic properties of malt as a flour supplement. Cereal Chem. (in this issue).
Munz, E., and Bailey, C. H.
1937 The effect of malted wheat flour upon certain properties of flour and dough. Cereal Chem. **14**: 445–457.

OBSERVATIONS ON ASH DISHES OF DIFFERENT MATERIALS [1]

W. J. EVA,[2] NANCY MILTON,[3] and W. F. GEDDES [4, 5]

(Read at the Annual Meeting, May 1938)

The importance of ash values in mill control has stimulated studies on methods for their determination; the majority of the papers deal with various methods of securing accurate results in a short time and there is relatively little information on the comparative values of ash dishes constructed of different materials.

Hertwig and Bailey (1925), using glycerol-alcohol solution as an ashing aid, found that platinum, porcelain, and silica gave identical results. Micka (1927) used platinum and porcelain dishes with various ashing methods and concluded that direct burning at high temperature in platinum without the addition of other substances was the most satisfactory. Davis (1932) in summarizing the results of collaborative studies on the magnesium-acetate-alcohol quick ash procedure reported that one of the participating chemists found silica [6] and porcelain dishes to give dependable results when new but these dishes depreciated rapidly with use at the high ashing temperature specified for this method. The American Association of Cereal Chemists (1935) prefer platinum or silica dishes for the A. O. A. C. official method; platinum is preferred for the "direct-weighing" and magnesium-acetate methods, and if silica or porcelain dishes are employed the inside surface should be smooth.

In preparing for a survey of the ash content of wheat originating in different districts, a preliminary study was undertaken to determine the most suitable ash dish and technique, the results of which are presented in this paper.

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[1] Contribution from the Grain Research Laboratory, Board of Grain Commissioners, Winnipeg, Manitoba. Published as paper No. 141 of the Associate Committee on Grain Research, National Research Council of Canada and Dominion Department of Agriculture.
Sub-committee report, 1937–38 Committee on Methods of Analysis.
[2] Assistant Chemist, Dominion Grain Research Laboratory.
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[4] Chief Chemist, Dominion Grain Research Laboratory.
[5] The authors wish to acknowledge the assistance of I. Levi in securing many of the data presented.
[6] Presumably opaque.

Experimental

Bailey (1937) in a study of the merits of several rapid methods reported the most satisfactory procedure to be a modification of the magnesium-acetate method, the results of which duplicated that prescribed by the A. O. A. C. Accordingly it was decided to employ the official A. O. A. C. and the A. A. C. C. magnesium-acetate method, using ash dishes of platinum (50 mm. × 20 mm.), transparent silica (55 mm. × 15 mm.), opaque silica (55 mm. × 15 mm.), porcelain (70 mm. × 13 mm.), and alumina (55 mm. × 15 mm.). With the exception of those made from platinum, the ash dishes were new and were used throughout the experiment without scouring.

For both ashing methods, three grams of the sample were employed with two methods of ascertaining the weight of the ash, (1) the weight of the dish before incineration of the sample was deducted from the weight of the ash plus dish and (2) the ash plus dish was weighed, the ash brushed out and the dish re-weighed. The latter method eliminates any possible error due to a change in weight of the ash dish during incineration but is subject to errors resulting from incomplete removal of ash. The ashings were carried out in an electric muffle furnace provided with pyrometer control. In the instance of the A. O. A. C. method the samples were incinerated overnight (approximately 16 hours) at 550° C., and for the magnesium-acetate method 45 minutes at 800° C. This latter temperature is 50° C. lower than that specified in Cereal Laboratory Methods but has been found necessary in order to reduce the tendency of certain samples to fuse. In other respects the technique outlined in Cereal Laboratory Methods was followed for both ashing procedures.

The experimental material comprised a finely ground sample of Western Canadian hard red spring wheat grading No. 1 Northern and a long patent flour experimentally milled from this class of wheat.

The ash tests were arranged in four series, one for each sample and ashing method. Each series involved the use of two dishes of each type daily for ten days employing the two methods of ascertaining ash weight; in the instance of the alumina dishes it was impossible to complete the series as they cracked before the conclusion of the experiment. The positions of the ash dishes in the muffle furnace were randomized.

Results

The available mean ash results for each sample, the ashing method, and the weighing procedure are recorded in Table I for each type of dish together with the standard errors for duplicates and replicates. In the standard errors for duplicates, differences between days are

TABLE I

ASH RESULTS—MEANS AND STANDARD ERRORS

Sample	Ashing method	Dish tare taken [1]	Transparent silica	Opaque silica	Platinum	Porcelain	Alumina
			%	%	%	%	%
MEAN ASH CONTENT							
Wheat	A. O. A. C.	Before	1.440	1.430	1.447	1.440	—
		After	1.437	1.430	1.440	1.433	—
Wheat	Magnesium acetate	Before	1.387	1.400	1.400	1.397	—
		After	1.387	1.400	1.400	1.383	—
Flour	A. O. A. C.	Before	0.507	0.503	0.507	0.503	0.503
		After	0.500	0.503	0.503	0.483	0.483
Flour	Magnesium acetate	Before	0.497	0.500	0.507	0.503	0.483
		After	0.497	0.500	0.507	0.483	0.427
STANDARD ERRORS For duplicates (within days)							
Wheat	A. O. A. C.	Before	0.020	0.025	0.029	0.020	—
		After	0.020	0.023	0.029	0.019	—
Wheat	Magnesium acetate	Before	0.018	0.015	0.017	0.020	—
		After	0.014	0.013	0.017	0.016	—
Flour	A. O. A. C.	Before	0.011	0.008	0.014	0.012	0.011
		After	0.006	0.009	0.009	0.007	0.008
Flour	Magnesium acetate	Before	0.014	0.015	0.014	0.026	0.019
		After	0.010	0.014	0.011	0.025	0.015
For replicates (day plus duplicate differences)							
Wheat	A. O. A. C.	Before	0.037	0.050	0.044	0.041	—
		After	0.038	0.051	0.041	0.043	—
Wheat	Magnesium acetate	Before	0.043	0.055	0.043	0.041	—
		After	0.045	0.056	0.043	0.045	—
Flour	A. O. A. C.	Before	0.017	0.023	0.012	0.017	0.014
		After	0.009	0.009	0.009	0.014	0.013
Flour	Magnesium acetate	Before	0.019	0.020	0.020	0.027	0.018
		After	0.015	0.017	0.016	0.026	0.019

[1] "Before" indicates that the dish tare was taken in the usual manner before incineration of the sample. "After" indicates that the dish tare was taken after incineration.

eliminated while those for replicates include the day differences. The increases in dish weights after each series and over the entire experiment are recorded in Table II. The discussion of the data which follows is based on the results of statistical analyses.

The alumina dishes proved to be quite unsatisfactory for ash work. Early in the experiment they developed hair-line cracks which rapidly enlarged and finally resulted in breakage. They showed large in-

TABLE II

CHANGE IN DISH WEIGHTS AFTER EACH SERIES OF TEN INCINERATIONS

Dish	Sample	Ashing method	Change in dish weight	
			Dish no. 1	Dish no. 2
			mg.	*mg.*
Transparent silica	Flour	A. O. A. C.	+0.2	+0.2
	Flour	Magnesium-acetate	+0.5	−0.8
	Wheat	A. O. A. C.	−0.4	+0.2
	Wheat	Magnesium-acetate	−0.2	+0.4
	Entire series		+0.1	0.0
Opaque silica	Flour	A. O. A. C.	−0.1	−0.7
	Flour	Magnesium-acetate	−0.5	−1.9
	Wheat	A. O. A. C.	−0.3	+0.6
	Wheat	Magnesium-acetate	−0.6	+0.5
	Entire series		−1.5	−1.5
Alumina	Flour	A. O. A. C.	+0.2	+2.8
	Flour	Magnesium-acetate	+18.1	+16.3
	Wheat	A. O. A. C.	+5.5	+5.0
	Wheat	Magnesium-acetate [1]	+6.4	+5.9
	Entire series		+30.2	+30.0
Platinum	Flour	A. O. A. C.	−0.1	+0.3
	Flour	Magnesium-acetate	+0.1	−0.4
	Wheat	A. O. A. C.	+2.4	+2.6
	Wheat	Magnesium-acetate	0.0	−0.1
	Entire series		+2.4	+2.4
Porcelain	Flour	A. O. A. C.	+4.1	+3.7
	Flour	Magnesium-acetate	+6.1	+3.7
	Wheat	A. O. A. C.	+2.9	+2.4
	Wheat	Magnesium-acetate	+4.6	+3.8
	Entire series		+17.7	+13.6

[1] Five incinerations only.

creases in weight throughout the experiments, the increase being most marked with the magnesium-acetate method. Being porous these dishes absorb some of the solution and accordingly give distinctly low results when the dish tare is taken after incineration of the sample.

The porcelain, transparent and opaque silica, and platinum dishes gave mean ash values on wheat and flour by both ashing methods which were not significantly different when the ash was computed from

the dish tare taken before incineration of the sample. The porcelain dishes, however, tended to give lower ash values, particularly by the magnesium acetate method when the dish tare was ascertained after incineration. As the experiments progressed the glaze of the porcelain dishes deteriorated and they increased considerably in weight. As with the alumina dishes the weight increase was greater when the magnesium-acetate method was used because of absorption of some of the solution into the body of the dish. For this reason, porcelain dishes give distinctly low results if the weight of ash is computed from the dish tare after incineration of the sample or if the ash is weighed directly on the balance pan.

The mean ash data indicate that silica, both transparent and opaque, and platinum dishes are equally satisfactory for the A. O. A. C. and magnesium-acetate methods and for the two methods of weighing. However, the silica dishes were new at the beginning of the experiment and it is our experience that after relatively short use in ashing hard red spring-wheat flours, opaque silica dishes show a decided tendency to scale. This results in weight decreases and makes it impossible to determine the ash by direct weighing. In the present experiments, both types of silica dishes gave standard errors which compare favorably with those for platinum. The platinum dishes gained considerably in weight and the inside surface took on a grey appearance.

A disturbing feature of these experiments is that, independently of the nature of the sample, type of dish, or method of ashing, relatively wide differences were found in the ash results obtained on different days. The existence of uncontrolled daily variations tends to invalidate comparisons between ash data obtained on different days; under such circumstances these day-to-day differences, in addition to duplicate error, must be taken into consideration. The maximum differences in mean ash content on different days were 0.113% and 0.027% for wheat and flour respectively. This situation is reflected in the higher standard errors recorded in Table I for replicates as compared with duplicates; in the former the differences between days are not taken into consideration. From these standard errors, the minimum significant differences in ash content for any two samples may be computed. If the level of significance is taken as twice the standard error, differences of 0.034% between two means of duplicate determinations conducted on wheat in platinum dishes by the magnesium-acetate method, with the customary method of ascertaining the dish tare, would be significant if the samples were incinerated together; if incinerated in different batches, the necessary difference for significance would be 0.086%. The corresponding values for flour

are 0.028% and 0.04%. Thus not only greater duplicate error but also greater daily variations occurred in the instance of wheat. The cause of these daily variations is probably associated with differences in atmospheric humidity; the ashing method and the method of taking the dish tare are not involved since the day differences were of similar magnitude in these instances.

Summary

Experiments were undertaken to determine the suitability of platinum, transparent and opaque silica, porcelain, and alumina dishes for ashing wheat and flour by the A. O. A. C. and magnesium-acetate methods. Ash weights were ascertained by taking the dish tare both before and after incineration of the sample.

Alumina dishes proved quite unsatisfactory. They were incapable of withstanding the high temperatures and progressively increased in weight particularly with the magnesium-acetate method. Because of their porosity, the dishes absorb some of this solution and accordingly give distinctly low results when the dish tare is taken after incineration of the sample.

Porcelain was less satisfactory than silica or platinum, particularly for the magnesium-acetate method. The glaze deteriorated, permitting partial absorption of the acetate solution into the body of the dish. This resulted in relatively large weight increases and rendered them unsuitable for the direct weighing of ash.

The silica dishes, both transparent and opaque, were equally satisfactory for both ashing methods and gave essentially similar mean values. Both types of silica dishes were more constant in weight than platinum. Experience has shown that opaque silica dishes tend to scale with continued use which results in weight decreases and renders it impossible to determine ash by direct weighing.

Regardless of the nature of the sample, type of dish, or method of ashing, relatively wide differences were found in the ash results obtained on different days. With platinum the minimum significant differences between the means of duplicates incinerated at the same time were 0.028% for flour and 0.034% for wheat. When the samples to be compared were incinerated on different days, the minimum significant differences were 0.040% for flour and 0.086% for wheat.

With flour the two ashing methods gave similar values in both silica and platinum dishes but the A. O. A. C. method gave significantly higher results for wheat.

Literature Cited

American Association of Cereal Chemists
1935 Cereal Laboratory Methods (3rd ed.). Published by the A. A. C. C.
Bailey, L. H.
1937 Some observations on methods of ashing cereal products. Cereal Chem. **14**: 120–128.
Davis, C. F.
1932 Report of collaborative studies on the magnesium-acetate-alcohol quick ash procedure. Cereal Chem. **9**: 431–437.
Hertwig, Raymond, and Bailey, L. H.
1925 Comments on "Glycerol as an Aid in Ashing Flour." Cereal Chem **2**: 38–41.
Micka, Jan
1927 Contribution to the determination of flour ash. Cereal Chem. **4**: 461–467.

TEMPERATURES ATTAINED IN BAKING PRODUCTS

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The temperatures within doughs or batters during their baking have been reported from time to time, but no single publication supplies this information on a variety of baking products. It is the purpose of this paper to present such data.

Catlin (1899) reported the internal temperature of baking-powder biscuits as attaining 217° F. by observing a thermometer through a glass in the oven door. Berg (1926) inserted a thermometer in bread after it was baked, whereas Hashitani and Sako (1932) did not state their method of following the temperature increase. Both workers found the maximum to be 98.5° C. Bailey and Munz (1938) determined the relation between the internal temperature of bread dough and the oven expansion. Hall (1930) used maximum thermometers in reporting maximum temperatures of 98° C. for layer cakes and 99° C. for loaf and sponge cakes. Barmore (1936) has reported an extensive study on the temperature of angel food cake when baked under variable atmospheric pressures, these representing variable altitudes. Thermocouples were used for following the heating up as well as the cooling of the cakes. Data taken from his curves are given here.

Altitude	Baking time	Maximum temperature
Sea level	45 min.	99.5° C.
5,000 ft.	60 min.	95°–96° C.
10,000 ft.	75 min.	90° C.

Results reported below on baking-powder biscuits, bread, and cake agree with those of previous investigators. Also given are the hitherto unreported results on pancakes, waffles, and muffins as well as inferred

results on doughnuts, wafers, crackers, and prepared dog-food biscuits. Crust temperatures are given in the instances of biscuits, muffins, and bread.

Experimental

Chromel-alumel thermocouples were imbedded in doughs or were suspended in position in batters. When it was found that a thermocouple had migrated from the approximate center of the baked product, its E.M.F. values were discarded before calculating temperatures. Thermostatically controlled gas and electric ovens were used where ovens were required for baking, but only the average results are given here. Certain adjustments of method were required with pancake and muffin batters.

Pancake batters, of the wheat-cake type, were poured on a griddle after at least three cakes had been fried, the thermocouple was held in place until it was supported by the coagulated batter, and at the appropriate time the pancake was turned. The temperatures are recorded in Table I as the average of three tests.

Waffle batters, the home type, were poured on a hot griddle with one thermocouple held in place at the center of a glass tube which rested in a groove half way along the radius of the circular baking iron. Another was placed in the batter and was observed after baking to be in the crust near the center. Data are presented in Table I from one

TABLE I

INTERNAL TEMPERATURE OF PANCAKES AND WAFFLES DURING BAKING

Time (seconds)	0	20	40	60	80	100	120	140	160	180
Pancake temp. (° C.)	–	88	93	98	99	100[1]	95	92	95	96

Time (seconds)	0	30	60	90	120	150	180
Waffle, couple in tube (° C.)	200	102	104	104	102	102[2]	111
Waffle, couple in crust (° C.)	—	104	110	118	138	154	173

[1] Pancake batter turned.
[2] Steam escape became negligible.

test only, but a duplicate test approximated these results. No doubt a higher temperature of the glass tube in contact with the griddle affected the thermocouple within it, but it is significant that the temperature approximated that of steam until steam escape became negligible, after which temperature rose sharply just before the baking was "done."

Baking-powder biscuit doughs were prepared with a soft-wheat flour, baking powder, salt, shortening, and milk. Thermocouples were inserted into the cut doughs from the sides.

Muffin batters were prepared using a two-cup-flour, two-egg home recipe. Thermocouples were supported from above.

White layer-cake batters were mixed according to a conventional baker's recipe (sugar-shortening cream) and a batter-cream recipe (shortening-flour cream). No appreciable differences in the rate of heating or temperature finally attained were noted with the two types of mixes, either in gas or in electric ovens.

Bread doughs were mixed with a straight dough formula. Thermocouples were inserted in the pan-proofed doughs just before inserting in the ovens. Records from centered thermocouples only were used.

Data obtained with centered thermocouples in baking-powder biscuit, muffin, cake, and bread doughs are given in Table II, together

TABLE II

INTERNAL TEMPERATURE OF BISCUITS, MUFFINS, CAKE, AND BREAD DURING BAKING

	Biscuits		Muffins		Cake	Bread
Baking time (min.)	15		25		30	32
Oven temp. (° F.)	450		400		365	450
Baking tin	Sheets, 2⅛" dia. cutter		2¼"–3" dia., 6-cup		8 × 1½" layer	1 lb. tinned
No. replica determinations	9		6		12	5
	Min.	° *C.*	*Min.*	° *C.*	° *C.*	° *C.*
	0	22	0	21	19	32
	1	28	2	34.5	34	32.5
	2	38	4	38.5	41.5	33
	3	47	6	46.5	47.5	32
	4	61.5	8	62	55	35
	5	79.5	10	80	63	39
	6	92.5	12	91.5	72.5	48
	7	97.5	14	97.5	78.5	55
	8	98.5	16	99.5	84	68
	9	99	18	100	87	77
	10	99	20	100	90	90
	11	99	22	100	94	94
	12	99	24	100	96	97
	13	99	26	100	97.5	98
	14	99	28	—	99	98.5
	15	99	30	—	99.5	99
	—	—	32	—	100	99.5

with information pertinent to conditions of baking. The data are shown graphically in Figure 1 as the "A" curves.

Data not given here were obtained by extending the time of baking a baking-powder biscuit at an oven temperature of 450° F. The center temperature attained 99° C. within 10 minutes and advanced to 100° after 15 minutes, and 101° between 23 and 25 minutes. An observable shrinking of the biscuit occurred after 20 minutes of baking. When freshly cut the interior of the biscuit was relatively firm. Within

a few minutes it was cool and crisp. A gradient of caramelization extended from the outside crust toward a lighter interior, the center being noticeably darker than a biscuit baked normally for 12 to 14 minutes. This experiment represents conditions existing within biscuits and crackers during their baking.

An extreme condition representing extensive dehydration and caramelization may be considered to take place in the baking out of prepared dog-food biscuits where 1 to 1¾ hours' baking time at 350° to 450° F. is required. Caramelization throughout the product indi-

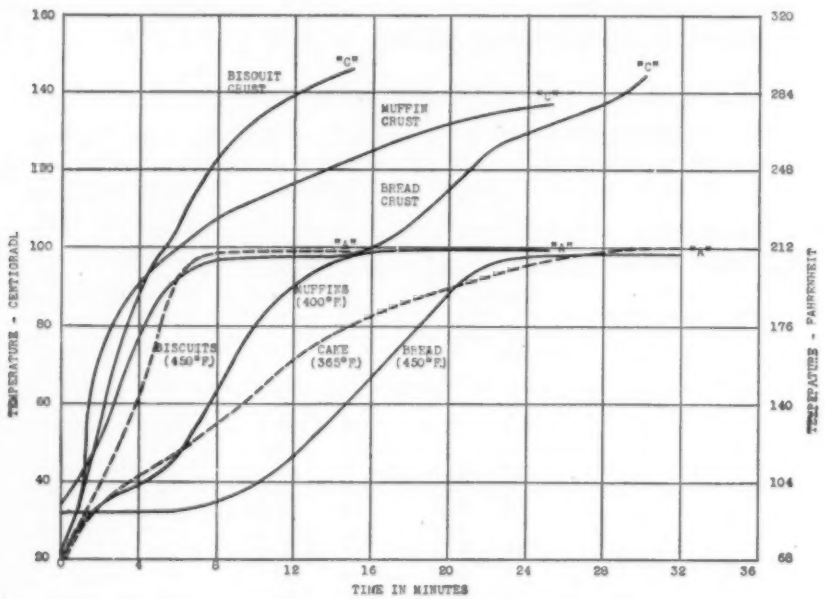

Fig. 1. *Temperature of baking products.* "A" curves—temperature taken at the center of the baking product. "C" curves—crust temperatures with thermocouples just within the crusts: baking-powder-biscuit top crust—1 mm., muffin side wall—2 mm., bread top crust—2 mm.

cates temperatures well above 100° C., which are similar to crust temperatures.

Crust temperatures are shown graphically in the figure as the "C" curves. Data are not recorded since these represent only one test in each case. Comparable results have been obtained, however, in repeated trials.

Discussion

The temperature attained within most doughs during baking is approximately that of boiling water. The rate at which this temperature is attained is dependent upon the size of the test piece, the oven

temperature, possibly the ingredients added, and the character and shape of the pan. The maximum temperature which will be attained will be variable with atmospheric pressure as demonstrated by Barmore.

Baked products characterized by a soft, spongy crumb do not attain internal temperatures over that of boiling water; only sufficient water is evaporated so that they will retain their cell structure when cooled. This is typical of the production of baking-powder biscuits, bread, cake, muffins, and, by inference, doughnuts.

Crisp, brittle, short-baked products such as waffles, wafers, crackers, and prepared dog-food biscuits require that their internal temperatures become greater than that of boiling water. The extent to which dehydration of dough colloids is carried is dependent on the baked product under consideration.

Crusts of baking products rapidly attain a temperature approximating that of boiling water, after which the temperature again rises as dehydration of dough colloids proceeds. The size of the test piece governs the rate of increase in temperature. The shape of the curves thereafter indicates that there is a slowing down of dehydration during caramelization of the crust, so that in a properly browned crust the temperature doesn't exceed 150° C.

Literature Cited

Bailey, C. H. and Munz, E.
1938 The march of expansion and temperature in baking bread. Cereal Chem. **15**: 413–419.

Barmore, M. A.
1936 The influence of various factors including altitude on the production of angel food cake. Cereal Chem. **13**: 71–78.

Berg, I. A.
1926 Controlling the cooling of bread. Baking Technology **5**: 206.

Catlin, C. A.
1899 Baking powders. Rumford Chemical Works, Providence, R. I.

Hall, H.
1930 A comparative study of the non-gluten constituents of soft and hard wheat flours. Cereal Chem. **7**: 270–280.

Hashitani, Y. and Sako, T.
1932 On the vitamin B content of bread. Cereal Chem. **9**: 115–116.

THE CUMULATIVE DEVELOPMENT OF BAKERY TASTE

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(Read at the Annual Meeting, May 1938)

During the past decade the baking industry has intensified its efforts to obtain a greater share of the potential cake and sweet-goods business. Although the major part of the bread business is now in the hands of commercial bakers, it is generally conceded that less than half of the cakes and sweet goods consumed in this country are commercially baked.

In an effort to discover the underlying causes for the apparent unwillingness on the part of many to accept commercially baked cakes in preference to those baked in the home, a number of surveys have from time to time been conducted. In these surveys, a large number of American homes were contacted in an effort to learn the reasons why commercially baked cakes were not acceptable. A study of the answers received from each survey discloses that one of the chief reasons for the lack of popularity of commercial cakes is "bakery taste." Here are a few typical replies received in one survey: "All bakers' cakes taste alike." "Bakers' cakes lack individuality." "Home-made cakes taste better."

Since bakery taste is one of the major reasons why more cakes are not sold, it would certainly seem that the development of bakery taste deserves more attention than it has received in the past. One thing is certain—the more familiar we become with the causes of bakery-taste development, the more readily shall we be able to correct the situation.

Let us assume that bakery taste is present to a greater or less degree in many commercial cakes. If this assumption is true, then it follows that these off-flavors may have been imparted to the baked goods in one of several ways.

First, there is the possibility that the ingredients may be responsible, and may introduce off-flavors, either through previous contamination or because they differ in quality and flavor from similar ingredients used in the home. Secondly, off-flavors may be introduced by the bakery environment, which is admittedly quite different from the domestic kitchen environment. Lastly, off-taste may be absorbed by the baked goods between the time they are finished and the time they are consumed.

Let us eliminate the possibility of the bakery taste's being caused by ingredients inferior to those used in the home. Personal observations by the author, which have been confirmed by many others in the baking field, indicate that bakery taste is often detected in merchandise made of ingredients which are actually superior in quality to those ordinarily sold for domestic use. Admittedly, some bakers have used raw materials which, because of their poor quality, definitely produced an off-flavor, but such cases are the exception. Thus, experience leads to a consideration of bakery environment as a more likely cause of the difficulty.

Historical

A survey of the literature on odors proved very helpful and tended to confirm our suspicions that bakery environment might be the principal factor in the development of bakery taste.

Zwaardemaker (1925), in a comprehensive treatment of the subject of odors, states that all odors must be volatile. Backman (1917) points out that odors must be soluble in water and also in lipoids. The necessity for this dual solubility in both water and lipoids is attributed by Backman to the fact that the cells of the smell-receptors in the nose are covered with a watery fluid, while they themselves contain lipoid granules. Therefore, if an odor is to be perceived by our sense of smell, it must be soluble in water in order to penetrate the layer of mucus, and must then be soluble in lipoids in order effectively to register on the receptors.

In an excellent review of the literature on food flavors, Crocker and Platt (1937) indicate that the only accepted tastes are sweet, sour, salty, and bitter. All other types of flavors register on the mind through the olfactory senses. Typical off-flavors which have been encountered under the general classification of bakery taste are, in most instances, odors, and not tastes. Sometimes these off-odors were introduced by the raw materials which had become contaminated by improper storage. At other times an examination of the raw materials indicated that they were free from off-odors. In many such instances it was possible to prove that these off-odors were absorbed either during mixing, baking, or subsequent handling.

That many food materials can pick up odors is generally recognized. Experience indicates that those ingredients which are finely divided and which contain either moisture or finely divided fats are most susceptible. Flour, cocoa, chocolate, coconut, nuts, eggs, shortening, sugar, and even paper and wrapping materials have, on various occasions, been shown to be the source of bakery taste.

Zwaardemaker (1925) also indicates that metals absorb odors and

are a potential source of flavor-contamination. Failure properly to wash equipment made of metal, therefore, should be kept in mind as a possible cause of bakery taste.

In speaking before the American Restaurant Association in 1929, "Oscar of the Waldorf" indicated that a well-appointed hotel kitchen should have separate ovens for different types of meat, fish, and game; otherwise, he stated, there is a tendency for "all meats to taste alike." This statement calls to mind the criticism so frequently heard: "All bakers' cakes taste alike." It appears that this problem is not confined to the baking industry, but is quite general.

When a woman bakes a cake, which she does at infrequent intervals, she is accustomed to pre-heat the oven. During this time, any odors therein tend to be dissipated. Under continuous baking, however, this is not possible, and it is only logical to expect that a batch of delicately flavored vanilla cake, for example, following on the heels of a batch of highly spiced cake might absorb unwanted odor, either during baking or afterward.

The identification of the cause or causes of bakery taste is at times most difficult. There is a minimum concentration or threshold for each odor, which varies with the individual. Zwaardemaker (1925) calls this threshold value (or minimum detectable concentration) the olfactic; and points out that each individual will exhibit a different olfactic for each type of odor. He also states that this threshold value is subject to nasal fatigue. Backman (1917) indicated that fatigue is caused by equalization of the concentration of odor in contact with the receptors and is only overcome when this concentration is increased.

Zwaardemaker (1925) points out also that odors vary in covering power. Thus, while lemon is a strong flavor and vanilla is a relatively weak one, vanilla greatly exceeds lemon in covering power. In the presence of certain odors of outstanding covering power, it is at times most difficult to trace another odor to its place of origin.

Another complicating factor is pointed out by Ruzicka (1920). He says that odors vary in characteristics as they change in concentration. Thus certain well-known flavors may smell entirely different when present in very small amounts. To make matters more complicated, there seems to be an interference factor at work. Nagel (1905) reports on some very interesting experiments in which vanillin was smelled by a number of subjects, who were then asked to smell a mixture of vanillin and coumarin in which vanillin predominated strongly. Because of the previous contact with vanillin, the various subjects could smell only coumarin. This phenomenon has been commented upon, and explanations have been offered by a number of

workers. Such experiments are the basis for the so-called chemical theory of odors. A discussion of this theory has been published by Durrans (1923).

Although the sense of smell in man is not particularly acute, being much more highly developed in other mammals such as the dog or the shark, and to a very marked degree in the insect world, it is surprising what small amounts of odoriferous substances may be easily detected through the sense of smell. By comparison, the smallest particle of matter that may be detected by other methods seems large. For example, it has been stated that the smallest subdivision of matter that is visible to the naked eye through the spectroscope is 1/1,400,000 milligram of sodium. Yet the nose can easily detect 1/250 of this weight of mercaptan. In tracing off-odors, therefore, we are attempting to identify and trace exceedingly minute amounts of odoriferous substances.

Experimental

Because of the prevalence of factory odors, our research bakery was completely air-conditioned, and all the doors and windows sealed with special weather-stripping. The air is completely turned over in a relatively few minutes, and one might expect ideal conditions, as far as freedom from off-odors is concerned. Repeated experiences indicated, however, that we were not so free from bakery taste as one might expect. On any number of occasions, baked products were carefully scored for flavor by a group in the research bakery and were assigned excellent ratings. Subsequently, samples were taken home and tasted there by the same group, and bakery taste was reported.

Examination of the raw materials disclosed the fact that they were not at fault. Packaging materials also were found to be without any odor contamination; therefore it seemed likely that the baked products were being contaminated by the air in the bakery.

In order to check this point, pie shells were made up and baked. Some were wrapped in cellophane and sealed as soon as they had cooled. Others were exposed for periods varying from one day to one week to the atmosphere of the bakery. These samples were then compared with the control by individuals in the research bakery, and were found to be only very slightly polluted by bakery taste. When another set of samples was examined at home by the same judges, they discovered a much greater contamination. It should be pointed out that at no time during this experiment would an individual be conscious of bakery or foreign odors in the research bakery. The atmosphere smelled sweet and free from pollution at all times.

Similar experiments involving whipped cream, boiled icing, cookies,

and pastry led to the same conclusions. Off-flavors were detected particularly in those bakery products that are mildly flavored. From theoretical considerations, whipped cream and boiled icings are ideally constituted for absorbing odors from the bakery. Whipped cream contains finely divided fat and considerable quantities of air. Boiled icing, on the other hand, contains no fat but still exhibits the same tendency to absorb off-odors. Mildly flavored cookies seem to be affected, whereas highly spiced cookies do not absorb sufficient off-odor to be noticeable.

Discussion

The development of bakery taste is, we believe, a cumulative process. When baked products or bakery raw materials are continuously exposed to atmospheres in which off-odors are below the normal olfactic, and therefore cannot be detected by the average individual, baked products can absorb these minute amounts in a continuous or cumulative fashion. If these food products are exposed for a long enough period, the concentration of off-flavor will build up to the point where it may be readily detected. The time necessary for this minimum detectable concentration to be reached will depend, of course, on the concentration of odors in the bakery. Where conditions are ideal, and the odors are present in amounts very much lower than the normal olfactic, it will be some time before the baked goods will exhibit bakery taste. Where the concentration of odors in the bakery is high enough to approach the olfactic, it may require only one or two days for baked products to accumulate a detectable off-flavor.

The value of adequate ventilation cannot be stressed too strongly, for ventilation is far more important than we have realized in the past. This is not only true of the bakery itself, together with the finishing and wrapping rooms, but is even more true of the ingredient storage rooms. When we realize that raw materials are kept in storage for weeks and even months, it is very easy to see how such ingredients as flour, for example, can absorb detectable amounts of off-flavors even when the air in the storage rooms seems to be free from all traces of off-odors. The ventilation conditions should be carefully studied, and the ideal situation will vary in almost every plant. If the bakery is located in a residential neighborhood where the likelihood of factory odors is negligible, it probably would be advantageous to insure the transfer of air from the outside through the ingredient storage and into the bakery. Such a situation would insure that the bakery odors could not contaminate the ingredients while in storage. Where factory odors predominate, the situation is, of course, just the reverse. Wherever possible, any deep-frying operations, including doughnut-

production, should be carried on in a separate room, with an exhaust fan so installed that the draft of air will be from the bakery into the frying room and thence to the out-of-doors through a stack that will prevent the frying odors from coming back into the bakery.

Dirt has been defined as matter which is "out of place." In a similar way, one might define "bakery taste" as flavors that are in the wrong place. There is probably no smell which is more appetizing than that of frying doughnuts, unless it be frying onions or frying ham, and yet these admittedly delicious and appetizing odors would be out of place when absorbed by whipped cream or a boiled icing, for example.

Improper installation and maintenance of equipment may also lead to development of bakery taste. When a complaint of an off-flavor in potato chips, characterized as "like kerosene" was investigated, it was found that a faulty oil-burner installation was the direct cause. It was not possible in this plant to detect a smell of fuel oil, because the concentration was below the olfactic. When chips were allowed to stand in the room for several hours, however, they absorbed sufficient fuel-oil odor so that a distinct off-flavor could be detected when the chips were tasted *outside the plant*. An examination of the chips inside the plant failed to disclose an off-flavor. This was unquestionably due to the effect of our constantly being subjected to the smell of fuel oil. This difficulty was corrected by enclosing the oil burner and feed pipes, thus providing for adequate ventilation from out-of-doors.

Suggestions for Detecting and Eliminating Bakery Taste

Carry on all taste tests outside of the plant, waiting for a few hours in order to allow the olfactory senses to become normal after prolonged exposure to the atmosphere of the bakery.

Carry on tests when foodstuffs are one or two days old in order to allow the concentration of off-odors to build up as they do under normal conditions of storage in the bakery.

When examining dry ingredients such as flour, starch, cocoa, and similar products, place a little in a cup or glass and add hot water. Stir vigorously, and smell the steam. This procedure tends to accentuate any off-odors and bring them to a concentration which is above the olfactic.

Pay particular attention to ventilation, not only in the bakery but in various ingredient and supply storage rooms.

Store wrapping materials (which are particularly sensitive) under as nearly ideal conditions as possible.

Keep the turnover of raw materials and bakery products at a maximum rate. This particularly applies to finished merchandise.

Keep the walls, ceilings, floors, and bakery equipment clean. If the greasy deposit which forms on the walls and equipment is allowed to remain indefinitely, it will pick up and hold off-flavors.

Wash pans and metal equipment frequently, since metals can absorb odors.

When placing food materials in the refrigerator, keep them covered wherever possible. Because the refrigerator is tightly sealed, it is frequently found to be a contaminating influence; therefore it should be cleaned and aired frequently. Even where this is done it is also advisable to cover sensitive ingredients in order to prevent the absorption of off-odors.

Literature Cited

Backman, E. L.
1917 Physiology of the sense of smell. Upsala Läkarefören. Förh. **22**: 319, 470.
1917 The strength of odorous substances and their solubility in water and oil. J. Phys. Path. Gen. **17**: 1, 4.
Crocker, E. C. and Platt, W.
1937 Food flavors—a critical review of recent literature. Food Research **2**: 183, 196.
Durrans, T. H.
1923 Odor and its origin. American Perfumer 405, 407.
Nagel, W.
1905 Handbuch der Physiologie des Menschen **3**: 613.
Ruzicka, L.
1920 Die Grundlagen der Geruchschemie. Chem. Zeit. **44**: 129, 131.
Zwaardemaker, H.
1925 L'odorat. Encyclopedie Scientifique.

BOOK REVIEW

Principles of Organic Chemistry. H. P. Stark, M.A. (Cantab.) Head of the Science Department, The Technical College, Kingston-on-Thames, formerly Lecturer in Chemistry, The Technical College, Bradford. Chemical Publishing Company of New York, Inc. viii + 664 pp. 58 figures. 14 × 25 cm. Price $5.00.

"The theoretical section covers the syllabuses of the Higher School Certificate courses of the Universities of Oxford, Cambridge, and London, and of various professional bodies.

"Students are apt to find organic chemistry difficult. . . . The difficulty of mastering first principles is increased by the introduction of complex compounds at an early stage, before their methods of preparation and characteristic properties have been described. An attempt has been made to avoid such complexity in the earlier parts of the book.

"The treatment does not claim to be exhaustive; the author has sought to curtail unnecessary material and to present the underlying principles as clearly as possible. . . ."

The text is divided into Part I, Theoretical (nineteen chapters of 486 pages) and Part II, Practical (nine chapters of 149 pages) and in addition a 21-page appendix of valuable material such as alcohol tables, vapor pressures, first aid, logarithms, answers to numerical problems, etc.

The theoretical part is divided into 14 chapters (376 pages) on aliphatic and five chapters (110 pages) on aromatic compounds. The first two chapters (51 pages) deal with methods of deriving molecular formulas. The treatment of the aliphatic compounds is the frequently used order beginning with the hydrocarbons, followed by halogen derivatives, alcohols and their derivatives, aldehydes, ketones, etc.

There are several charts and tables which give comparisons and contrasts between different types of compounds or which show applications of various substances. The Geneva system of naming is not used, being avoided by avoiding naming or mentioning the more complex compounds with which it would be needed. No mention is made of the electronic formulas.

Several experiments are given in the body of the text. In the Practical part, some of the experiments given are qualitative, a number of preparations are given, and a chapter is devoted to the quantitative estimation of a variety of things, for example urea, uric acid, sugar, fats, soaps, and hardness in water. A chapter is also devoted to general tests for carbohydrates, proteins, alkaloids, etc.

There is a good list of questions at the end of each chapter, many of which are taken from various Boards of Examiners and from Civil Service examinations. The printing is good and the editing has been well done.

R. C. Abbott

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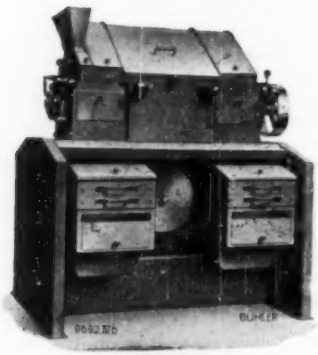

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